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BIOLOGICAL BULLETIN

COMPARATIVE PHYSIOLOGY OF THE INVERTEBRATE HEART.[1]

With Plates IV. to VIII.

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I. The Innervation of the Heart.

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Before proceeding to describe the ganglia and the nerves that make connection with the cardiac apparatus particularly in the molluscs it seems necessary to make an explanation regarding the nomenclature to be followed. There is no little confusion in the terminology applied to the different ganglia and nerves connected with the viscera and the respiratory organs in the mol-

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luscs. This is, in a great measure, due to the difficulty in making out the homology between the ganglia and their connectives in the different groups, but also to the fact that names have been invented with sole reference to the *position* of the ganglia, not to mention the far-fetched homologies that have been made out between the molluscan and the vertebrate nervous systems. Thus Alder and Hancock (1842) apply the terms "cerebro-spinal" and "sympathetic" to the nervous system of the gasteropods. Chéron (1866) endeavors to make out an analogy between the stomato-gastric nervous system in the cephalopods and the sympathetic system in the vertebrates. And Ransom (1884) applies the term "vagi" to the two nerves from the pleuro-visceral ganglia to the cardiac and the branchial ganglia of *Octopus*. The discrepancies in these homologies between the molluscan and the vertebrate nervous system are so considerable that the introduction of the terms "cerebro-spinal," "sympathetic," "vagus," etc., serves rather to confuse. Scarcely more commendable is the introduction of names of ganglia with reference to their position only. Thus Bottazzi and Enriques (1901) reject the commonly accepted names "cerebral," "pleural" or "pallial" and "pedal" ganglia for the œsophageal nervous complex of *Aplysia*, and substitute the terms "dorsal," "lateral," and "ventral" ganglia respectively. These names fit the conditions in *Aplysia* admirably; but how will they apply to *Pleurobranchæa*, in which the pleural and the cerebral ganglia are fused into one ganglionic mass situated dorsally, or to the Doridida, in which both the cerebral, the pleural, and the pedal ganglia are fused into one ganglionic mass situated dorsally with reference to the œsophagus? It is plain that if we were to follow the principle suggested by Bottazzi and Enriques, different names would have to be invented to fit the conditions in the different subdivisions of the gastropods, and frequently even for different genera within the same group, because the position of some of these ganglia is subject to considerable variation. This objection applies also to the term "subanal," which is used by Lacaze-Duthiers (1859) for the ganglion commonly known as the visceral ganglion in the Haliotidæ.

Bottazzi and Enriques reject the names, cerebral, pleural or

pallial and pedal on the ground that they are physiological misnomers, and such they are to some extent. The same objection may be raised to the names "visceral ganglia" and "visceral nerves" or "pleuro-visceral commissures," because these ganglia innervate other structures besides the viscera and because some of the visceral organs are innervated by nerves from other ganglia. The stomato-gastric nervous complex of the cephalopods and the gasteropods is as much "visceral" as the two pleuro-visceral connectives with their peripheral complex of ganglia. This is recognized by Haller (1882), who calls the buccal ganglia of the prosobranchs the "anterior visceral ganglia." But this requires new names for the visceral nerves in the cephalopods and for the pleuro-visceral connectives in the gasteropods. The principle of naming the different ganglia and nerves with reference to the organs innervated by them is the correct one, as it will insure the greatest possible uniformity of names in the different groups. But our knowledge of the molluscan nervous system does not yet allow such a revision of the terminology. And for that reason I will make use of the names visceral nerves and visceral ganglia, inadequate and misleading as they are, rather than burden the literature with additional terms, which in all likelihood would prove to be but makeshifts.

In the dissections no attempts have been made to work out the innervation of other organs besides the heart, except in so far as the nerves to the cardiac apparatus are involved. In the figures the nerves to the various visceral and pallial organs are indicated for the purpose of orientation. The results of the dissections by aid of a good hand lens have not been supplemented by histological methods. Frequently nerves could be followed to the base of the auricles or on to the aorta but not on to the auricles and the ventricles themselves, although stimulation of the nerves showed that the nerves entered these organs. I have no doubt that in such cases the nerves could have been traced on to the cardiac musculature by aid of histological methods.

1. *The Lamellibranchs.* — Nerves to the cardiac apparatus of the bivalve molluscs have been described by Quatrefages (1849) for *Terredo*, and by Hancock and Embleton (1852) for *Mya truncata*.

Quatrefages describes two pairs of exceedingly small ganglia situated in the posterior part of the pericardial cavity. Nerves from these ganglia reach the base of the auricles and enter the musculature of these organs. Other branches from the ganglia ramify in the pericardium. A small nerve connects each pair of ganglia with the large pleuro-visceral ganglion on the ventral surface of the posterior adductor muscle. Hancock and Embleton describe "two small elliptical ganglia attached to the anterior and under part of the branchial (visceral) ganglia and united by commissures. These send filaments to the ovary and to the ventricle of the heart." The ganglia and the nerves are not figured, nor is it stated in what way the nerves reach the heart. List (1902) in his recent monograph of the *Mytilidæ* of the Gulf of Naples makes no mention of the innervation of the cardiac apparatus. He describes nerves to the renal organs and the reproductive glands, but follows them only a very short distance from the pleuro-visceral ganglia. Dogiel (1877) has described nerve cells in the auricles and at the auriculo-ventricular junctions of *Pecten* and *Anadonta*, but he does not make out their connection with any nerves and ganglia outside the heart.

My own work was done on *Mytilus californianus*, *Mya arenaria*, *Tapes staminea* and *Platydon cancellatus*. In *Mytilus* (Pl. IV., Fig. 1) I found a series of small ganglia that probably correspond to the cardiac ganglia described by Quatrefages in *Terredo*. A small nerve is given off from the dorsal side of each visceral ganglion (*vg*). These nerves (4) run dorsally for a short distance close to the anterior surface of the posterior adductor muscle and then turn anteriorly, reaching the dorsal body wall through a portion of the reproductive gland. At this point two slender branches are given off; the lateral branch (5) can be followed anteriorly in the body wall into the base of auricle, but it cannot with certainty be traced on to the auricular musculature. The other nerve (6) can be followed anteriorly on to the pericardium near the median line. The main branch of the nerve takes a median course in the body wall towards the median line, but before reaching the median line it bifurcates, one branch passing posteriorly (7) and one anteriorly in the dorsal body wall. At the point of bifurcation there is a small ganglion (8, 9). The branch that takes the

anterior course enters a small ganglion situated dorsal to the hind gut at the junction of the body wall with the pericardium (10). The two ganglia are connected by a tiny nerve. Several small nerves are given off anteriorly from the ganglia to the pericardum and the gut, but I was unable to trace any of these branches on to the musculature of the ventricle. There are individual variations as regards the place of branching of the nerves and the two sides are usually not symmetrical. If the ganglia on the course of these nerves correspond to the "cardiac ganglia" of Quatrefages, there is this difference to be noted that in *Mytilus* the ganglia are not situated in the pericardial cavity and that branches of the nerves can be traced to the base of the auricles but not on to the ventricle. The two nerves in *Mytilus* are probably more of dorsal mantle nerves than of cardiac nerves, for even where the nerves ramify in the pericardium, the pericardium and the body wall are fused into one. In most of the gasteropods the renal and the cardiac nerves are united in one common trunk. In *Mytilus* the renal nerves are given off from the cerebro-visceral commissures (11) and they can be followed throughout the whole length of the kidney at the base of the gill, but I was unable to trace any of the branches on to the auricles or the ventricle.

The visceral nervous complex of *Mya* (Pl. IV., Figs. 2 and 3) differs somewhat from that of *Mytilus*. The visceral ganglia are fused into one with enlargements at the point of origin of the branchial nerves, and there are in most cases slight ganglionic enlargements at the cerebro-visceral commissures a little anterior to their union with the visceral ganglion. One or two small nerves pass laterally from each commissure to the ganglionic protuberance at the point of origin of the branchial nerves (Fig. 2, 3). From the commissures close to the visceral ganglion two small nerves take their origin (Fig. 2, 4). After an anastomosing with the nerves from the commissures to the sides of the visceral ganglion these nerves take a postero-lateral course on the surface of the kidney between the commissures and the branchial nerves. Branches of these nerves can be traced into the substance of the kidney and to the base of the auricle. Posterior to the point of origin of the branchial nerves another pair of tiny nerves are given off to the lateral surface of the kidney (Fig. 2,

6). From the dorsal side of the visceral ganglion two nerves are given off similar to those described in *Mytilus*. One of these nerves passes to the posterior adductor muscle and the dorsal body wall of the anal region; the other nerve (Fig. 2, 3) also sends branches to the adductor muscle but the main branch passes dorsally to the body wall where it takes an anterior direction and can be followed into the dorsal wall of the pericardial cavity. On its course branches are given off to the kidney, the aorta, and the rectum (Fig. *3*, 3 and 4). This nerve is evidently homologous with the dorsal mantle nerve in *Mytilus* (Fig. 1, 4).

In *Tapes staminea* the visceral ganglion is situated in the angle made by the posterior adductor muscle and the adductor muscles of the foot. The ganglion gives rise to five pairs of nerves, viz., the posterior mantle nerves (1), the nerves to the siphon (2), the great osphradio-branchial nerves (4), the cerebro-visceral commissures (*cvcom*), and a pair of tiny nerves to the posterior adductor muscle (3). In the figure these last nerves are turned to the side so as to be represented in the same plane, as they turn in a dorsal direction almost at right angles to the other nerves. The commissures and the branchial nerves run parallel and close together for a distance of about one centimeter from the ganglion, and then the branchial nerves turn laterally and anteriorly into the osphradium and the gills. At the level of the pericardial cavity the commissures penetrate the reproductive gland, to which they send fibers. Close to the visceral ganglion each commissure gives off a small nerve (5) which runs parallel with it for a short distance and then turns laterally to enter the kidney and the heart. These nerves may be designated the reno-cardiac nerves, and are evidently homologous with the nerves marked 4 in Fig. 2. The renal organs occupy the space between the adductor muscles of the foot and the pericardial cavity and extend along the base of the gills. The nerves branch so extensively in the kidney that I was not able to follow any one branch directly on to the auricles, but the physiological evidence is conclusive that these nerves send branches to the heart. The nerves to the ventricle enter that organ solely through the auricular walls, as severence of the anterior and the posterior aortæ together with the rectum at either end of the ventricle does not interfere with

the influence of the nerves on the heart, but after the auricles have been severed, leaving the other connections intact, stimulation of these nerves or the visceral ganglion has no further effect on the heart.

Platydon differs from *Tapes* in that the reno-cardiac nerves (3) are given off from the visceral ganglion instead of from the commissures. A small ganglion (4) can also be made out on these nerves at the base of either auricle.

In *Venus*, *Cardium*, *Hennites* and *Pecten* the cardiac nerves were not worked out in detail, but the heart of these lamellibranchs is innervated from the visceral ganglion or the cerebro-visceral commissures just as in *Mya* and *Tapes*, as will be shown by physiological experiments in a subsequent paper. This is evidently the plan of the reno-cardiac innervation in all lamellibranchs. The cell bodies of the cardiac nerves are probably situated in the visceral ganglion or ganglia, the giving off of the nerves from the cerebro-visceral commissures instead of from the ganglion directly is evidently only a case of the fibers following the course of the commissures for some distance before turning laterally to enter the kidney and the heart. While it is certain that the heart of these molluscs is innervated from the visceral ganglion, the nerves entering the heart at the base of the auricles, the exact course of these nerves through the renal organs as well as in the heart itself remains to be worked out. This must be left to biologists who have more suitable material, like Tridacna, at their disposal.

Young (1881) studied the effect on the heart of the stimulation of the visceral ganglion in the lamellibranchs *Mya*, *Anadonta* and *Solen*. He gives no definite description of the course of the nerves from the visceral ganglion to the heart. He mentions two tiny nerves which he could trace from the ganglion on to the pericardium and the rectum, thinking that these were the cardiac nerves. From his statement that branches from these nerves pass to the rectum he evidently refers to nerves similar to those indicated by me in *Mya* and *Mytilus* as dorsal mantle nerves (Fig. *1*, 6, 10; Fig. *3*, *2*); but the physiological evidence goes to show that these nerves do not enter the heart.

2. *The Chitons.*—The chitons present a more diffused nervous

system than any other gasteropod. There are no definite ganglia, the nerve cells being scattered all along the main nerves or "nerve-cords." So far as I am aware, nerves to be cardiac apparatus of these animals have not been described. Haller (1883) describes nerve cells and nerve fibers in the walls of the auricles, but he does not make out the connection of these nerve fibers with any nerve or nerve-cord outside the heart. My own work was done on *Cryptochiton stelleri*. This species was selected because of its large size, individuals being frequently found that measured 30 cm. in length. From the lateral and ventral side of the œsophageal nerve ring (Pl. IV., Fig. 6) proceed two pairs of nerve-cords. The median pair passes posteriorly into the foot, the lateral pair runs posteriorly in the mantle in a sinus between and slightly dorsal to the efferent and afferent gill sinuses, to unite at the posterior end of the mantle. These are the pleuro-visceral cords. From these cords numereus nerves are given off, some taking a lateral and ventral direction to the gills, others passing dorsally into the mantle and median into the wall of the body cavity. The nerves vary in size, but they are all very small and branch soon after leaving the cord, so that it is difficult to follow them for any great length. In the region of the heart one and sometimes two of these tiny nerves can be followed in the wall of the efferent gill sinuses and on to the auricles (Fig. *6*, *1*). On their course these nerves branch like the rest so that only a small division of the nerve reaches the auricle and this branch cannot be followed in the auricular musculature for any considerable distance. These are, however, probably not the only nerves that reach the auricles from the pleuro-visceral cords. The auricles are closely attached to the body wall along the side or base, Haller (1883) even claiming that the auricular musculature shades imperceptibly into that of the body wall. Posterior to the opening of the efferent gill sinuses into the auricles, several nerves can be followed to within less than 1 mm. of the place of their attachment to the body wall (Fig. *6*, *2*). It seems therefore probable that the auricles are supplied, not with one but with several nerve filaments from the nerve cords. No nerves could be traced to the heart or the pericardium from the pedal cords.

3. *The Diatocardic Prosobranchs.* — Nerves to the heart of the diatocardic prosobranchs have been described by Lacaze-Duthiers (1859), Haller (1883), Bouvier (1889), and Illingworth (1902). Lacaze-Duthiers describes in *Haliotis* four nerves from the pleuro-visceral connectives and the visceral ganglion to the pericardium. He calls these cardiac nerves, but he does not trace them to the auricles or the ventricle. Haller finds that the heart of *Fissurella*, *Turbo* and *Trochus* is innervated from two different ganglia. He traces nerves from the branchial ganglia along the efferent gill sinuses to the auricles, while the ventricle is innervated by a nerve from the visceral ganglion that reaches the ventricle along the aorta. Bouvier describes similar nerves to the auricles and the ventricle of *Patella*, *Nerita* and *Helicina*, but he does not figure the course of the nerve from the visceral ganglion to the ventricle nor does he state whether the nerve enters the ventricle along the rectum or at the aortic end. Illingworth states that the cardiac nerves described by Haller and Bouvier are also present in *Lucapina*.

My own work was done on *Lucapina crenulata* and *Haliotis cracherodii*. In *Haliotis* (Pl. V., Fig. 7) two nerves (7 and 8) can be followed from the visceral ganglion or from the pleuro-visceral commissures close to the ganglion, to the renal organ and the base of the auricles. The nerves were not traced on to the auricular musculature, but I have physiological evidence that motor nerves from the visceral ganglion supply the auricles and it is probable that they reach the auricles in the trunk of the nerves 7 and 8. The visceral ganglion also gives off two nerves to the pericardium, one (9) passing to the dorsal, the other (10) in the ventral wall. The latter nerve sends one or two branches on to the aorta, but I did not succeed in tracing these branches on to the ventricular musculature. It can be shown physiologically, however, that motor nerves from the visceral ganglion enter the ventricle at the aortic end, and it is probable that these nerve fibers reach the organ in the ventral pericardial nerve. The innervation of the ventricle of *Haliotis* is consequently the same as that described by Haller for *Fissurella*, *Turbo* and *Trochus*, a nerve from the visceral ganglion entering the ventricle at the aortic end. I did not succeed in tracing any nerves from the

branchial ganglia to the auricles, nor did I find any physiological evidence of their existence. If such a nervous connection exists, the nerves are evidently only sensory in function. The auricles are, however, innervated from the visceral ganglion.

The nervous system of *Lucapina* differs from that of *Haliotis* chiefly in the presence of two large ganglia, the intestinal ganglia, on the course of the pleuro-visceral commissures. From the posterior side of the visceral ganglion (Pl. V., Fig. *8*) a comparatively large nerve passes in a dorsal direction in the wall of the basibranchial sinus towards the rectum, but before reaching the rectum the nerve bifurcates, one branch passing on either side of the rectum to take an anterior course in the walls of the afferent gill sinuses. From the ganglion and from the nerve between the ganglion and the point of bifurcation two or three small nerves are given off (3, 4), and branches from these nerves can be followed into the pericardium. Illingworth states that he could trace some of these branches into the musculature of the ventricle along the rectum. I was not able to do so, and the physiological evidence is to the effect that the nerve enters the ventricle at the aortic end and not along the rectum. After the bifurcation, each nerve gives off a small branch that can be followed to the base of the auricle (5). From the gill ganglia several nerves are given off, but the only one that concerns our present inquiry is the tiny nerve described by Illingworth as passing posteriorly in the walls of the efferent branchial sinus, branching and anastomosing on its course, to finally end in the musculature of the auricle. I was able to follow the nerves (9) for some distance towards the base of the gills, but not on to the auricles. Stimulation of the efferent gill sinus does not influence the auricles, so that if this nervous connection, described by several investigators for so many species, is present in *Lucapina* the nerves are in all probability only sensory in function. The auricles of *Lucapina*, like those of *Haliotis*, are innervated from the intestino-visceral commissures or the visceral ganglion, as shown by stimulation of the commissures. The exact course of these auricular nerves remain to be worked out.

4. *The Monotocardic Prosobranchs.* — Nerves to the heart of the monotocardic prosobranchs have been described by Haller

(1882) and Bouvier (1889). Haller worked on *Murex trunculus*. In this mollusc there are three visceral ganglia. From the middle and largest ganglion a nerve passes along the aorta to the ventricle, while the auricle is innervated from the nerve plexus in the efferent gill sinus in the manner described by the same author for *Fissurella*. Bouvier worked on several genera but he describes the innervation of the heart particularly in *Cyclophorus tigrinus* and *Triton variegatum*. In *Cyclophorus* he describes a nerve to the ventricle only. "A gauche du ganglion (viscéral), la commissure viscérale donne naissance à un gros nerf cardiaque qui suit un instant l'aorte anterieure et penetre ensuite dans le ventricle. On le voit tres bien entrer dans l'organe sur la preparation que j'ai conservie, et je pense qu'il n'est pas un type plus commode pour l'étude de l'innervation du coeur" (p. 77). In *Triton* there are two visceral ganglia connected by a commissure. This commissure gives rise to three small nerves, one of which passes to the branchial vein, another to the auricle, and the third to the ventricle. This last nerve is given off by the commissure close to the right visceral ganglion. It runs for some distance in the pericardium and enters the ventricle at the aortic end.

My own work was done on *Natica lewissii* and *Sycotypus canaliculatus*. In *Natica* (Fig. 9) the right pleuro-visceral commissure is much stouter than the left. It enters the large branchial ganglion on the left side, which gives rise to four nerves, namely a comparatively large nerve to the osphradium and gill (3), a smaller one to the floor of the pallial cavity (2), a somewhat larger branch to the gill (4), and finally a small branch to the left visceral ganglion (5). On the course of this last nerve is a small ganglion (7) which sends a tiny nerve to the ventral wall of the pericardium (6). The two visceral ganglia are connected by a commissure. The right ganglion is much larger than the left. From the former two and sometimes three nerves take their origin, the largest of which divides immediately after leaving the ganglion, the main branch passing to the reproductive glands and the viscera (8), the smaller branch turning forward and laterally into the ventral wall of the pericardium (9). Branches from the two pericardial nerves (6 and 9) can be fol-

lowed to the base of the auricle, to the junction of the aorta with the ventricle, and into the kidney. This arrangement is also born out by the physiological experiments. Each of the two nerves sends fibers to the auricle and to the ventricle by the aortic end. There is some individual variation as regards the point of origin of the genital and the reno-cardiac nerves from the right visceral ganglion. In some specimens the nerves do not leave the ganglion close together as indicated in Fig. 9, Pl. V., but the ganglion is distinctly bilobed and the genital nerve takes its origin from the anterior lobe near the entrance of the commissure. The innervation of the heart in *Natica* thus agrees in main with that of *Murex*, *Cyclophorus* and *Triton* as described by Haller and Bouvier. There is complete agreement in the innervation of the ventricle, nerves from the visceral ganglion or from the visceral commissure enter the ventricle at the aortic end. If the auricular nerve from the nerve plexus in the branchial sinus, as described by Haller in *Murex*, is present in *Natica*, it is only sensory in function, because stimulation of the branchia ganglion or the nerve in the efferent gill sinus (4) has no effect on the auricle.

The innervation of the heart of *Sycotypus* (Fig. 10) presents some differences from that of *Natia*, and *Sycotypus* being the larger animal the nerves and the ganglia can be worked out with greater accuracy. The right pleural ganglion gives rise to three nerves, two of which (*10*) unite to enter the osphradial and branchial ganglia. The third constitutes the left pleuro-visceral commissure. On the course of this commissure some distance from its entrance into the visceral ganglion in a slight ganglionic swelling giving rise to a tiny nerve which ramifies in the floor of the branchial chamber along the line of attachment of the gill. One of its branches turns posteriorly towards the base of the gill and the auricle, but I could not trace it on to the auricle itself. A similar tiny ganglion on the left commissure (*11*) gives rise to a nerve which enters the rectum and adjoining structures. The left visceral ganglion, the smallest of the two, gives rise to three or four small nerves, which ramify in the connective tissue making up the anterior and ventral wall of the pericardial cavity. One branch (*2*) can be traced to the base of the auricle, another

branch (3) communicates with the ganglion on the ventriculo-aortic junction (8). The large visceral ganglion on the right side also gives rise to several nerves, one of which (5) can be followed with the unaided eye through its course in the pericardium till it enters the ganglion on the ventriculo-aortic junction. Branches from nerve 7 enter the renal organ. Nerve 4 enters the reproductive gland and the liver. Apart from nerve 5, which plainly enters the ventricular ganglion and is therefore, a cardiac nerve, there are several branches from nerves 4 and 6 that ramify in the pericardium. Whether any of these branches enter the auricle at its base I am unable to make out.

The ganglion on the ventriculo-aortic junction is large enough to be seen by the naked eye, and by the aid of a dissecting lens nerves can be traced from the ganglion for some distance on the ventricle (9).

There is no evidence of nerve-fibers passing from the branchial ganglion to the auricle. Either end of the heart is supplied with nerves from the visceral ganglia, and this is born out by the physiological experiments. The commissure between the two visceral ganglia is partly ganglionic and pigmented like the visceral ganglia themselves.

5. *The Tectibranchs.* — In his description of the nervous system of *Pleurobranchus* Lacaze-Duthiers (1857) does not come to any definite conclusion regarding the innervation of the heart. "Sur l'oreillette, on trouve des filets nerveux évidents, mais qu'il m'a été impossible, vu leur delicatesse, de les suire jusqu'à leur origin" (p. 285). But he believes that the auricular nerve makes connection with the right peripheral nerve from the visceral ganglion. This nerve is called by him the genital nerve. Dogiel (1877) describes in *Aplysia* a nerve to the auricle from the left and posterior side of the visceral ganglion. Vayssière (1879) has figured and described the nervous system of *Gasteropteron*, *Doridium*, *Philene*, *Scaphander* and *Bulla*, all of which belong to the family Bullididæ. Of the visceral organs he describes the innervation of the œsophagus, the stomach and the reproductive organs, but he makes no mention of the innervation of the heart. Ransom (1884) has shown that the left side of the visceral ganglion of *Aplysia* gives origin to two instead of one nerve as figured by Dogiel.

The anterior of these two nerves supplies the reproductive organs. The other nerve runs towards the gill, where it bifurcates, one branch entering the gill; the other "appears to end in the pericardium near the origin of the auricle." In his recent monograph of the Aplysidæ Mazzarelli (1893) describes and figures three nerves from the left side of the visceral (called by him deutovisceral) ganglion. Of these nerves the one given off furthest posterior "manda un sotil ramo alla membrana viscerale" (p. 108); but no reference is made to the innervation of the heart. Pelseneer (1893) has described the nervous system of *Bulla striata* and *Pleurobranchæa mekeli*. In *Bulla* he dot not figure the peripheral nerves from the two visceral ganglia (called by him supra-intestinal and abdominal ganglia), and consequently not the innervation of the viscera and the heart. In *Pleurobranchæa* he describes two nerves from the visceral ganglion. The one on the right side passes to the gill. The left nerve is called the genital nerve and the small ganglion on its course the genital ganglion, but the nerves peripheral to this ganglion are not described and consequently not the cardiac innervation. The most recent observations in this field have been make by Bottazzi and Enriques on *Aplysia* (1901). These authors describe a nerve from the right side of visceral ganglion to the aorta and from the left side of the ganglion "un nerf cardiaque, qui donne un rameau au nephredion et un qui va au second estomac triturateur (?)" (p. 122).

The presence of ganglion cells in the heart of *Aplysia* has been affirmed by Dogiel (1877) and denied by Ranson (1884).

My own work was done on *Aplysia californica*, *Bulla globosa* and *Pleurobranchæa californica*. In *Aplysia* (Pl. VI., Fig. 11) the pleural or pallial ganglia of the œsophageal complex give rise to two stout nerves, the pleuro-visceral commissures, which run free in the body cavity of the neck to enter into connection with the large visceral ganglion situated in the body cavity anterior to the visceral mass. From the right and posterior side of this ganglion proceeds a stout nerve (1) which enters the osphradium and the roof of the gill chamber. The left and anterior side of the ganglion gives rise to three nerves. The most anterior one (5) enters the mantel and the dorsum of the anal region. The middle nerve

(4) supplies the gill. These two nerves reach their destination by a circuitous route, passing ventral to the heart. The third nerve (3) is the visceral nerve par excellence, because it sends branches to the liver, the reproductive glands, the heart and the renal organ. Arising from the postero-lateral side of the ganglion this nerve takes a direction towards the aortic sinus and the pericardium, but before it reaches these structures it bifurcates. The branch that passes ventral to the aortic sinus (7) gives off a branch to the liver (10) and one or two very small branches to the aortic sinus (12), while the main trunk (11) follows an artery to the reproductive glands. The other nerve (6) takes a postero-lateral direction in the ventral wall of the pericardial cavity. On reaching the postero-lateral side of the pericardial cavity it takes a median direction parallel with long axis of the heart. Several small branches are given off by the nerve to the pericardium, and at the level of the auricle a tiny branch (9) is sent forward in the ventral wall of the pericardium and on to the auricle, while the main branch (8) enters the kidney. The nerve on the aortic sinus (8) could not be traced on to the ventricular musculature with certainty, but we shall presently see that nerves do enter the ventricle at the aortic end and that these nerve fibers leave the visceral ganglion in the trunk of the posterior nerve of the left side of the ganglion (3). From the posterior side of the visceral ganglion one or two small nerves (14) are given off which ramify in the musculature and connective tissue of the dorsum immediately adjoining the ganglion and cannot be followed for any distance. From the right pedal ganglion arises a small nerve (13) which connects with the right side of the visceral ganglion. The corresponding nerve of the left side does not come into connection with the visceral ganglion but enters directly into the musculature of the dorsum a little anterior to the pericardial cavity.

Electrical stimulation of either of these two nerves produces none of the effects caused by the stimulation of the pleuro-visceral connectives.

The visceral nervous system of *Bulla* (Pl. VI., Fig. 13) differs from that of *Aplysia* mainly by the presence of five or six ganglia on the course of the nerves. The left commissure has a small ganglion on its course before reaching the main visceral ganglion.

At this point a small nerve (2) is given off to the dorsum of the neck. In some specimens the ganglion is not situated at the point of origin of the nerve but nearer the visceral ganglion. The right visceral ganglion gives off peripherally one stout nerve (5), which enters the osphradium; here it divides and sends branches into the gill and to the roof of the gill chamber. The left ganglion gives off a corresponding nerve (6), which also bifurcates soon after leaving the ganglion. At the point of bifurcation is a small ganglion. One of the branches enters the inferior pallium, the other the posterior dorsum. Either visceral ganglion gives rise to another nerve (3, 4), much smaller than the one that takes its origin at the posterior end. These nerves unite in a small ganglion (7) situated to the left of the two main ganglia. This ganglion is called the "genital ganglion" by Vayssière, which is not a well chosen name, because the nerve that leaves the ganglion supplies not only the reproductive glands but also the renal organ and the heart. The nerve (8) that is given off by the ganglion soon bifurcates and at the point of bifurcation is another ganglionic enlargement (9). The main branch (10) takes a lateral direction and enters the genital glands, the other passes towards the pericardial cavity. This branch of the nerve sends a small filament on to the aortic sinus (11), while the main trunk passes posteriorly and median in the pericardium ventral to the heart, finally entering the kidney (14). Several small branches are given off to the pericardium, one of which can be followed to within one or two mm. of the base of the auricle, but not on to the auricle itself. The branch to the aortic sinus ramifies on the sinus making almost a complete ring at the ventricular junction. A branch can be followed on to the ventricle. In *Aplysia* I was unable to trace a nerve on to the ventricle, in *Bulla* I failed to trace the nerve to the auricle; but the physiological evidence is conclusive that fibers from the nerve (8) enter the auricle of *Bulla* at its base. The connection is probably with one of the tiny branches in the ventral wall of the pericardium.

In *Pleurobranchæa* (Pl. IV., Fig. 12) the pleural ganglia constitute the postero-lateral portion of the supra-œsophageal ganglion. The first pair of nerves that proceeds from the posterior and lat-

eral side of the ganglion enters the mantle, the nerve on the right side also sending branches to the gill. From the second pair of lateral ganglionic protuberances are given off three nerves, one from the right side and two from the left. The posterior one on the left side, the larger of the two, enters the posterior part of the mantle of the same side. The other nerve (*lvn*) follows the course of the cerebro-pedal and pedal commissures towards the right side, where it takes a posterior direction and joins the visceral ganglion situated anterior and to the right in the body cavity. The corresponding nerve on the right side (*rvn*) is much stouter and is probably homologous to the two nerves given off from the corresponding ganglionic protuberance on the left side. Peripherally the visceral ganglion gives off two nerves. The nerve on the right side (1), the largest of the two, passes posteriorly in the body cavity at the margin of the mantle and the foot and enters the gill. The left nerve passes dorsally towards the pericardial cavity, a little in front of which it enters a small ganglion (3), designated by Lacaze-Duthiers as the "genital ganglion," which is certainly a misnomer, because the ganglion supplies other organs besides the reproductive glands. Five nerves are given off by this small ganglion. One of these (10) passes on to the anterior aorta and follows it along its ventral side to the aortic sinus, where it bifurcates, one branch (12) passing along the lateral, the other (13) along the posterior aorta. In large specimens a small ganglionic swelling can be made out at the place of branching (11). A tiny branch (14) is given off by this ganglion to the aortic sinus, but I did not succeed in tracing it on to the ventricle. The smallest nerve of the five bifurcates soon after leaving the ganglion, sending one branch (9) on to the ventriculo-aortic junction, the other branch (8) is lost in the connective tissue surrounding the ganglion. The nerve given off from the median part of the ganglion (5) is larger. It passes posteriorly to the auriculo-ventricular junction where it penetrates the muscular bands which make up the ventral wall of the pericardium and enters the auricle. Just as it enters the auricle a branch is given off to the ventral wall of the pericardial cavity. The main part of the nerve can be followed in the wall of the ventricle almost around the entire organ. It breaks up in several branches

(7) which pass towards the base of the auricle and perhaps into the venous sinuses between the auricle and the gill. In the largest specimens one or two of these branches can be traced to the base of the gill. This nerve is probably homologous to the nerve in *Aplysia* and *Bulla* which runs in the ventral wall of the pericardial cavity and supplies the heart and the renal organ. Parallel to this nerve runs an extremely small branch (6), sometimes given off from the ganglion, sometimes from the auricular nerve itself. The branch enters the pericardium. And lastly, the ganglion gives off a comparatively large nerve which takes a posterior and median direction and enters the genital glands (4).

6. *The Nudibranchs.* — The innervation of the viscera in *Doris tuberculata* has been described in great detail by Hancock and Embleton (1852). Four visceral nerves take their origin from the right and ventral side of the supraœsophageal ganglionic mass. One of these nerves sends branches to the renal organ, the gill, the vesicle ventral to the pericardial cavity (believed by these investigators to be a "portal heart"), and to two ganglia situated "on the apex of the ventricle." Besides these two ganglia on the ventricle they describe a great number of small ganglia on the peripheral course of the visceral nerves. Pelseneer (1893) designates two small nerves from the abdominal ganglia in the two Doridida *Polycera* and *Goniodoris* as "reno-cardiac nerves," but he does not follow them to their entrance of these organs.

My own work done on the three Doridida, *Montereina nobilis*, *Triopha carpenteri* and *Triopha grandis*. *Montereina* is the largest nudibranch at my disposal, and for that reason best adapted for working out the cardic innervation and for isolating the cardiac nerves for the physiological experiments. In this mollusc (Pl. VI., Fig. 14) the œsophageal chain of ganglia as well as the greater part of the visceral ganglia are fused into one supraœsophageal ganglionic mass, the more primitive arrangement of the ganglia being indicated by the presence of the stout commissure which passes under the œsophagus and connects both sides of the ganglion. From the right and ventral side of this composite ganglion near the origin of the œsophageal commissure a nerve (*vn*) is given off, which takes a posterior direction ventral to the two posterior nerves to the mantle. On reaching a branch of

the anterior aorta it follows the latter in a median direction towards the hind gut, where it bifurcates. The smaller branch (1) continues in the median direction along the artery and, passing ventral to the hind gut, it can be followed to the left ventral side of the aortic sinus. The main branch passes posteriorly on the left side of and close to the hind gut. At the level of the ventricle it gives off a small branch (2) which passes in a median direction dorsal to the hind gut and enters a small ganglion situated on the aortic sinus (6). From this ganglion one or two branches can be traced on to the aorta and another on to the ventricle. This ganglion evidently corresponds to the two ganglia described by Hancock and Embleton in *Doris tuberculata* as situated on the "apex of the ventricle." The main branch of the nerve continues posteriorly along the gut to the anal region. On this course the nerve gives off a branch (3) to the ventral wall of the pericardium and probably also to the renal organ. The terminal branches of the nerve (4, 5) pass to the base of the auricle and to the gill. There appears to be a small ganglionic swelling on the nerve before it breaks up into the gill branches. I was not able to follow the nerve into the auricle, but the physiological evidence show clearly that nerve fibers from this nerve enter the auricle at its base.

In *Triopha carpenteri* (Pl. VI., Fig. 15) the central nervous system is fused into one ganglionic mass situated dorsal to the œsophagus, just as in *Montereina*. From each postero-lateral horn of the ganglion proceeds one nerve which innervates the mantle (1, 2). From the posterior and right side of the ganglion, ventral to the origin of the right mantle nerve, four small nerves take their origin. The destination of two of these (5, 8) could not be made out with certainty, but the other two nerves supply the viscera. The nerve furthest to the right (7) enters a small ganglion (*gi.*) situated on the vagina. This ganglion sends branches to the copulatory organs and the reproductive glands. The other nerve (6) passes posteriorly in the median line dorsal to the visceral mass. On this course the nerve gives off several tiny branches which cannot be followed for any distance from the nerve on account of their minuteness. The largest branch probably enters the intestine and the liver (9). The main trunk

of the nerve is continued posteriorly to within a short distance of the large forward loop of the intestine where it bifurcates, the smaller branch (10) passing along the anterior aorta ventral to the intestinal loop, the larger branch (11) turning slightly to the right reaches the base of the auricle and the gill along the rectum on the right side of the pericardial cavity. I could follow the smaller branch (9) along the aorta to within 2 mm. of the ventricle, but not on to the ventricle itself; nor could I trace any branch from the other division of the nerve on to the auricle. Electrical stimulation of the nerve 7 shows that fibers from this nerve reach both auricle and ventricle. The course of this nerve (7) is very similar to that of the "visceral" nerve in *Montereina* (Pl. VI., Fig. 14, *vn*). The left side of the supraœsophageal ganglion gives rise to a small nerve (4) which innervates the stomach, and probably also the digestive glands and the intestine.

Triopha grandis attains to a considerably greater size than either of the two foregoing Dorididæ, but it is much less abundant. I have obtained only a few specimens of this nudibranch at Monterey Bay during the months of July and August, when it comes into shallow water to breed. I was therefore unable to make any physiological experiments on the heart and the heart-nerves, but sufficient material was at my disposal to work out the cardiac nerves anatomically. This appears to be essentially the same as that of the previous species, only that the greater size of this mollusc enables one to follow the nerves to their terminations with greater accuracy. Nerves 1 and 2 (Fig. 16) take their origin on the posterior and lateral sides of the brain and pass laterally and posteriorly on the dorsal surface of the visceral mass close to the visceral envelop, on this course sending numerous branches to this envelop and to the dorsum. At the level of the sinous passages from the gill to the auricle the two nerves enter two small ganglia situated in the angle made by the base of the auricle and the rectum (9). These ganglia are joined by a commissure. From each ganglion a tiny nerve passes dorsally and anteriorly evidently entering the pericardium and the auricle. Stouter branches pass posteriorly into the gill. These branches have several small ganglia on their course.

Nerve 3 takes its origin slightly dorsal to the right branchio-

mantle nerve and takes a course lateral to it. On reaching the anterior or cephalic artery the nerve bifurcates, the smaller branch (4) continuing posteriorly to the genital organs. The final destination of this nerve was not determined, but it does not come into relation with the heart. The main branch follows the artery, ventral to it, in a median direction, and at the level of the forward loop of the intestine it bifurcates, the smaller branch passing dorsal to the gut, its branches ramifying in the visceral envelop and on the surface of the liver. None of its tiny rami can be traced to the region of the paricardium and the heart. At the place of bifurcation is usually a small ganglionic swelling (5). The main branch continues posteriorly along and ventral to the artery and can be followed on to the junction of the aortic sinus with the ventricle. Branches from this nerve pass to the main arteries and probably follow them peripherally just as in *Pleurobranchæa*. In large specimens the branch to the ventriculo-aortic junction (7) can be traced on to the ventricle. This is, therefore, one of the cardiac nerves. The heart is thus supplied with nerve-fibers from nerve 3 and in all probability also from nerve 2 (and 1 ?) just like the two foregoing Doridida, in both of which nerves enter the heart at the aortic as well as at the auricular ends.

7. *The Pulmonates.* — Ransom (1884) has described nerves to the auricle and the ventricle of *Helix:*

"From the median protuberance on the œsophageal ganglionic mass comes off almost from the middle line a couple of visceral nerves. The larger of the two, the left nerve, runs along the aorta towards the ovisperm duct, to which it gives off a branch. It then divides, and one part follows the aorta into the ventricle while the other goes to the kidney, where it ramifies and some branches are continued to the origin of the auricle" (p. 327).

Curiously enough, Ransom figures the ventricle as being situated anterior to the auricle. Plate (1898) describes four nerves from the visceral (pleural) ganglion in the Janellidæ. Of these nerves three enter the pallial complex and the fourth supplies the renal organ. "Er versorgt in erster Linie die Niere. Ob er, ausserdem, wie wahrscheinlich ist, den Herzbeutel und das Herz, villeicht auch die Athmrören innerviert, bleibt weitern Untersuchungen zu Feststellung vorbehalten" (p. 256).

My own work was done on the two slugs, *Limax maximus* and *Ariolimax columbianus* and on the snail *Helix* (*Arionta*). In *Limax* (Pl. VII., Fig. 17) the pedal and pallial ganglia are fused into one ganglionic mass situated ventral to the œsophagus and connected with the supraœsophageal ganglion by a commissure on either side of the œsophagus. This pallio-pedal ganglion is pierced by the cephalic artery; the portion of the ganglion dorsal to the artery is the pallial ganglion proper. This dorsal portion of the ganglion gives rise to four nerves. The two lateral ones (1, 2), which are the stoutest, pass to the pallial complex and the mantle. Besides the mantle nerve, the left side of the ganglion gives rise to two smaller nerves, one of which takes a posterior direction close to one of the adductor muscles and ramifies in the dorsum at the place of attachment of these muscles. The other nerve (*vn*) runs posteriorly a little to the right of the copulatory organ. On this course a branch is given off to the salivary gland. At the level of the bifurcation of the aorta the nerve enters a small triangular ganglion (4), situated in the connective tissue close to the reproductive gland. The ganglion gives rise to three nerves. The smallest one of these (5) passes to the reproductive gland. A larger branch (7) takes a posterior and median direction along the posterior artery to enter the liver and the intestine. The largest branch (6) takes a dorsal and anterior direction towards the kidney, but before reaching that organ it bifurcates, both filaments entering the ventral side of the kidney. The nerve that follows the posterior artery (7) gives off two very tiny branches to the aorta, but I was not able to follow them on to the ventricle. Nor was I able to trace the nerves that enter the kidney to the musculature of either the ventricle or the auricle, although a branch can be followed to within 2 mm. of the base of the auricle. The physiological experiments prove, however, that fibers from the visceral nerve enter the auricle and the ventricle at the base of the auricle.

The pallio-visceral nervous complex in *Ariolimax* (Pl. VII., Fig. 18) does not differ essentially from that of the slug just described. The left mantle and pallial nerve (2) enters the pallial complex close to the rectum. The visceral nerve (*vn*) is given off dorsal and a little to the left on the median protuberance of the gang-

lion. The nerve takes a posterior direction along the cephalic aorta for a distance of about 3.5 cm., when it divides in two, one branch continuing in the posterior direction along the posterior aorta to the liver and the intestines, the other turning dorsally towards the kidney. Here the nerve bifurcates, both branches entering the kidney at its ventral side, a little to the right of the exit of the aorta through that organ. By aid of a strong dissecting lens a filament from one of these renal nerves can be followed on to the ventriculo-aortic junction. I was not able to trace any nerve branch to the auricle, although stimulation of the visceral nerve shows that nerve fibers enter the heart of this slug both at the aortic and the auricular ends. The dissection reveals no ganglion at the point on the visceral nerve where the reno-cardiac nerve is given off, corresponding to that in *Limax*.

The nervous system of *Helix* (Pl. VII., Fig. 19) resembles that of the slugs very closely. The pallial ganglion gives rise to four nerves, the lateral pair (1, 4) entering the pallial complex and the mantle. The right member of the median pair (2) goes to the pallial complex, but it cannot be traced to the auricle or the kidney, and stimulation of the nerve does not affect the heart. The left member (3) of the median pair is the visceral nerve proper. On its posterior course it gives off a branch to the copulatory organs (5), and a little further posterior another small branch which probably enters the lung (6). At the point of origin of this branch there is a slight ganglionic swelling on the nerve. At the level of the ventricle the nerve bifurcates, one branch continuing in the posterior direction into the reproductive gland (9). The other branch (7) turns to the right and before entering the kidney gives off a filament (8) which takes a posterior direction, probably reaching the stomach and the intestine. The nerve can be followed into the kidney without any difficulty, but I was not able to trace any of its branches through the kidney and into the auricle, as Ransom has described and figured it, although I have physiological evidence that such connection is made. Before the nerve enters the kidney a small filament is given off to the aorta and the pericardium, but I could not trace it on to the ventricular musculature, although the physiological evidence is conclusive that nerve fibers enter the ventricle at the aortic end.

8. *The Cephalopods.* — Nerves to the cardiac apparatus of the cephalopod molluscs have been described by Chéron (1866), Fredericq (1878), and Ransom (1884). Chéron worked on *Eledone*, *Octopus*, *Sepia* and *Loligo*. In the *Octopoda* four nerves are given off from the sub-œsophageal or pleuro-visceral ganglion. Two of these nerves pass to the stellate ganglia in the mantle. The median pair of nerves takes a posterior direction on the ventral surface of the liver and innervates the rectum, the cardiac apparatus and the gills. At the level of the auricles is found a small ganglion on each nerve, and from these ganglia nerves are given off to the auricles and the systemic ventricle. Further on their course each nerve sends a branch to the small ganglion situated on the gill ventricle, while the main nerve trunk enters the large ganglion at the base of the gill. According to Chéron the arrangement is the same in the Decapoda, with the exception of the innervation of the auricles and the systemic ventricle. Chéron describes a commissure between the two visceral nerves a short distance in front of the systemic ventricle. At the junctions of the commissure with the visceral nerves are found ganglionic swellings, which he considers homologous with the ganglia on the visceral nerves of the Octopoda which innervate the auricles and the systemic ventricle. Fredericq and Ransom worked on *Octopus*, but apart from their physiological results they add nothing to the anatomy of the cardiac nerves as given by Chéron. Neither of these investigators make any mention of a commissure between the two visceral nerves similar to that described by Chéron for the decapods, but Fuchs (1895) figures such a commissure in *Octopus*.

My own work was done on *Octopus punctatus*, *Loligo pealii* and *Ommastrephes illecebrosa*. The innervation of the cardiac apparatus in *Octopus* I found in all essentials the same as described by the authors just referred to, and the reader is referred to the figures given by them. In *Loligo* I found some relations not described by Chéron, and the cardiac innervation of this cephalopod will therefore be described with some detail. From the median lobe of the pleural ganglion (Pl. VII., Fig. 20) proceeds a stout nerve, which as it penetrates the cranial cartilage separates into two flattened branches running close to each other in

a posterior and ventral direction through the liver, and on reaching its ventral surface they pass posteriorly between the liver, and the viscero-pericardial sac. At this point in their course a small branch is given off from each nerve to the cephalic vena cava, which lies just ventral to the nerves. The two branches unite into one (11) which enters the vein, branching out anteriorly and posteriorly in its muscular walls. A similar nerve to the vena cava is described by Chéron for *Eledone*, *Sepia* and *Loligo*, but he figures it as coming off from the pleural or pallial ganglion a little anterior to the point of origin of the visceral nerves. At the level of the anus the two visceral nerves diverge, taking a more ventral direction and half encircling the vena cava. The circle is made complete by branches given off from each nerve to the rectum and the ejaculatory duct of the ink gland and a commissure between the two sets of nerves (1, 2, 3). The commissure passes between the vena cava and the rectum. One of the branches from the left nerve (2) can be followed in the viscero-pericardial sac near the median line almost to the posterior end of the body cavity. On its course numerous branches are given off to this visceral envelop, but none of these can be traced to any of the cardiac organs. The visceral nerves continue posteriorly close to the vena cava, and at the level of the ink gland, to which branches are given off (4), they take a lateral and dorsal direction, passing dorsal to the auricles and the branchial sinuses to a ganglion at the base of the gill (*bg.*). Before the nerve reaches this ganglion, that is, about 3 mm. central to it, a branch (6) is given off to a small ganglion situated dorso-median on the gill ventricle near the junction of the ventricle with the afferent gill vein (9). From this ganglion a small nerve passes on to the dorsal surface of the auricle (7). At the point of entrance of the nerve into the auricle a small ganglionic enlargement can be made out on the nerve (10). There is some individual variation at this point. Instead of one branch from the visceral nerve to the ganglion on the gill ventricle there are sometimes two or three, one of which usually proceeds from the gill ganglion. A branch may also run past the ganglion on the gill ventricle and join the branch from this ganglion to the auricle. I was not able to follow the nerves that enter the

auricles on to the systemic ventricle. The ganglion at the base of the gill measures 1 mm. in diameter in the largest specimens and the ganglia on the gill ventricle and the auricle are only one third as large, so that it is difficult to locate them by dissection, especially as they are imbedded in the connective tissue that envelops these organs. From the gill ganglion a small branch passes to the retractor muscle of the gill (8). In the male a branch from the left visceral nerve innervates the penis (5). At the level where the branches are given off to the ink gland (4) commissural fibers extend across from one visceral nerve to the other. This commissure would correspond to that described by Chéron for the decapods. If branches are given off to the systemic ventricle by the visceral nerves at this point as physiological experiments indicate, and as Chéron found to be the case, the systemic heart of *Loligo* has a double nervous supply, as nerves unquestionably pass to the auricles along with the nerve or nerves to the ganglia on the gill ventricles. Chéron figures the ganglia which supply the nerves to the gill ventricles as situated on the trunks of the visceral nerves, and not on the ventricles themselves, as I found to be the case in *Loligo*.

The dissection of the smaller branches of the nerves in *Loligo* is very difficult, because the nerves, the connective tissue and the muscular tissue have in life very nearly the same color and transparancy; and in addition, minute arteries, which are hardly distinguishable from the nerves, run in the larger nerve trunks and ramify with the smaller nerve branches.

The specimens of *Ommastrephes illecebrosa* obtained at Woods Holl are more than twice the size of *Loligo*. The species of *Ommastrephes* on the coast of California (*O. californica*) attains a length of from 3 to 3½ feet, but the only specimen of this large species at my disposal was so badly preserved that the finer branches of the nerves could not be told apart from the smaller arteries. The main course of the visceral nerves in *Ommastrephes* (Fig. 21) is the same as in *Loligo*. The commissure (*vc.*) between the two visceral nerves a little anterior to their lateral divergence from the vena cava to enter the gills, is very distinct. The commissure gives rise to a nerve (2) which follows the vena cava in a posterior direction to enter the ventricle. The nerve divides

and anastomoses extensively on its course along the vena cava and sends numerous branches to the renal veins. In *Loligo* Chéron figures a nerve as passing to the auricles from either visceral nerve at the level of the commissure in a manner similar to that in *Octopus*. In *Ommastrephes* there is no distinct ganglionic enlargement at the junctions of the commissure with the visceral nerves. The nerve passing to the heart takes its origin from the commissure and not from the visceral nerves directly, and this nerve enters the ventricle, not the auricle.

At the level of the auricles each visceral nerve bifurcates, the smaller branch of each (3, 4) taking a median direction on the ventral and posterior surface of the systemic ventricle. Near the origin of the posterior artery these branches are connected by a tiny commissure, after which they unite into one nerve-trunk (10). This follows the posterior aorta for a little distance to finally enter the reproductive organs. There is a slight ganglionic enlargement at the place of bifurcation.

The branches to the gill ventricles and the auricles (5) leave the visceral nerves close together a little distance before these nerves enter the gills. The nerve to the gill heart (6) runs posteriorly along afferent gill sinus and penetrates the substance of the gill heart at its junction with this sinus. The auricular nerves are very slender. In some specimens they are given off, not from the visceral nerves directly, but from the branch to the gill ventricles. There is a distinct ganglionic enlargement on the visceral nerves at the point of origin of the nerves to the gill ventricles.

This ventral visceral nervous complex is connected with the cerebro-gastric nervous system by a commissure (8) from the left visceral nerve to the gastric ganglion (*g.*). This nervous connection has not, to my knowledge, been noted before in the cephalopods. I was unable to make it out with a certainty in *Loligo* owing to the difficulty of distinguishing between the smaller arteries and the finer nerve-branches.

The systemic heart of *Ommastrephes* (and in all probability also *Loligo*) is thus furnished with a double nervous supply similar to that of the gasteropod molluscs, fibers from the visceral nervous complex entering the systemic heart both at the auricular

and at the aortic ends. The presence of the communicating branch between the gastric ganglion and the visceral nerves is of interest in view of the statement by Bottazzi and Enriques (1901) that the cerebro-gastric commissure in the œsophagus contains accelerator fibers to the systemic heart (*Octopus*).

9. *The Decapod Crustaceans.* — Considerable work has been done on the innervation of the heart in the decapod crustaceans. Lemoine (1868) describes, in the crab, a branch to the anterior and dorsal surface of the heart from the gastric nerve. The gastric nerve makes direct connection with the cerebral ganglia and the œsophageal commissures, but not with the thoracic ganglion. The existence of this cardiac nerve or nerve of Lemoine was confirmed on anatomical as well as physiological grounds by Young (1878, 1879), Plateau (1878, 1880), and Moquart (1883). Plateau states that "l'existence du *nerf cardiaque* de Lemoine est incontestable." Both Young and Plateau conclude that this cerebro-cardiac nerve is accelerator in function, and that in addition to this accelerator nerve the heart is also supplied with inhibitory nerves from the thoracic ganglion. Important modifications and additions to these results have been made by Jolyet and Viallanes (1892) and by Connant and Clarke (1896). Jolyet and Viallanes found that in the crab (*Carcinus*) both the accelerator and inhibitory centers for the heart are situated in the thoracic ganglion, and that when the cerebro-thoracic commissures are severed, stimulation of the cerebral ganglia or the gastric nerve has no effect on the heart. Nor could they find the "nerve of Lemoine" anatomically. In the pericardium they found a plexus of nerve cells and nerve fibers, from which proceed three nerves on either side in a ventral direction towards the thoracic ganglion, but the connection of these nerves with the ganglion was not made out. This pericardial nerve plexus had previously been described by Dogiel (1878). The results of Jolyet and Viallanes were confirmed by Connant and Clarke on the crab (*Callinectes*). They were equally unable to obtain any anatomical or physiological evidence of the existence of the "nerve of Lemoine," but they traced the connection between the thoracic ganglion and the six nerves which Jolyet and Viallanes had described as entering the pericardial nerve plexus. These

cardiac nerves take their origin dorsally on the thoracic ganglion close to the origin of the recurrent cutaneous nerve, the nerve to the third maxilliped, and the nerve to the first ambulatory appendage respectively. The nerves that reach the heart in the trunk of the recurrent cutaneous nerve, that is, the anterior pair of cardiac nerves, were found to be inhibitory in function, the two posterior pairs to be accelerator. Bottazzi (1901) has confirmed the results of Jolyet and Viallanes and Connant and Clarke as regards the presence in the thoracic ganglion of both accelerator and inhibitory centers to the heart.

Nerve cells in the walls of the crustacean heart have been described by Berger (1876), Deszö (1878), Young (1878), Dogiel (1877, 1894), Plateau (1880), and Pagoschwa (1890). Deszö states that the ganglion cells are especially abundant in the posterior half of the dorsal wall.

The results of Jolyet and Viallanes and of Connant and Clarke on the crab appeared to me so conclusive that I did not take the time to work out the cardiac nerves in the available material of this group (*Cancer, Brachynotus, Epialtus*), but instead I confined the anatomical work to the large spiny lobster (*Palinurus*), and it may be stated at the outset that the cardiac nerves in this decapod have nearly the same relations as given by Connant and Clarke for *Callinectes*. The dorsal side of the large thoracic ganglion gives rise to six pairs of small nerves (Pl. VIII., Fig. 22, 1 to 6). The anterior pair takes its origin near the roots of the nerves to the third maxilliped. On leaving the ganglion the nerves run alongside (but not in the same sheath as) the recurrent cutaneous nerves for some distance in an anterior, lateral and dorsal direction. The recurrent nerves turn posteriorly after passing around the anterior processes of the endophragmal skeleton, but their smaller companions (1) pass through the foramina between the two anterior endostermites. Reaching the inner surface of the endoskeleton at this point the nerves take a dorso-lateral direction ventral to the anterior flexor muscles of the abdomen, and on reaching the point of attachment of the extensor muscles the nerves pass posteriorly on these muscles and finally ramify in them posterior to the level of the pericardial cavity. At the level of the pericardial cavity each nerve gives off a branch (7) to the

pericardium and the heart, the slender filaments reaching the dorsal and posterior side of the heart along the suspensory ligaaments. The nerves that are given off from the thoracic ganglion near the roots of the first ambulatory nerves (2) take a dorsal direction through the opening between the second and the third endosternites and pass ventral and lateral to a greater bulk of the flexor muscles than is the case of the anterior pair. On reaching the extensor muscles the nerves run posteriorly on their surface, ventral to the anterior nerves, and at the level of the anterior end of the heart each nerve gives off a small branch (8) to the plexus of arteries and suspensory ligaments at the anterior end of the pericardial cavity. In very large specimens the nerves can be followed along the suspensory ligaments to the dorsal side of the heart.

The other nerves from the dorsal surface of the thoracic ganglion (3 to 6) turn dorsally through the openings of the subsequent endosternites and ramify, with the arteries, on the flexor and extensor muscles that are situated dorso-laterally in the thoracic cavity. I could not trace any of their branches to the heart, nor does stimulation of these nerves effect the heart. That the two anterior pairs of nerves really send fibers to the heart is further shown by stimulation of these nerves. The anterior pair contains inhibitory, the second pair accelerator fibers. In *Callinectes* Connant and Clarke found that the inhibitory fibers reach the heart in the recurrent cutaneous nerves (*rcn.*). This is not the case in *Palinurus*. Branches from the posterior rami of these nerves can be followed close up to the pericardial cavity, but not on to the heart itself, or on to the suspensory ligaments, and in no instance did stimulation of these nerves effect the heart. The course of the cardiac nerves in the plexus of suspensory ligaments and small arteries in and surrounding the pericardiac cavity is not easily made out, because the nerves, the arteries and ligaments have nearly the same color and transparency.

10. *The Arachnids.* — To the recent paper on the innervation of the heart of *Limulus* by Patten and Redenbaugh (1899) I have only one item to add, viz., the connection of the inhibitory nerves with the heart. The heart and the heart-nerves of this interesting arthropod are shown in Fig. 23. The ganglion cells

and the main nerve-trunks are confined to the dorsal side of the heart. The large nerve-trunk on the dorso-median side of the heart is in reality an elongated ganglion, being made up of nerve-fibers and ganglion cells. This nerve-cord or ganglion extends the whole length of the heart, but it is largest in the fourth, fifth and sixth segments, tapering thence both in the anterior and the posterior direction. There are relatively few ganglion cells in the nerve-cord of the first and second segments. Besides this elongated ganglion on the dorso-median side of the heart there are two nerves running parallel with it and lateral to the ostia. These are the lateral nerves. The nerves branch and anastomose extensively, especially in the middle region of the heart. In large specimens the nerves can be followed with the naked eye up to the first, and posteriorly to the last segment, but they are stoutest in the fourth to the sixth segments. There appears to be no ganglion cells on the course of these nerves. A very complex system of connectives extends between the median nerve-cord and the lateral nerves. On the whole, one pair of these connectives are given off from the median nerve-cord at the level of each pair of ostia, the connectives being the largest in the fourth, fifth and sixth segments. The connectives usually branch extensively on the dorsal side of the heart before joining the lateral nerves. The median nerve-cord, the lateral nerves as well as the main connecting branches are large enough to be easily made out with the naked eye in the living heart.

The nervous complex on the dorsal side of the heart is partly covered by the elastic connective tissue fibers, but these can be removed without any injury to the nervous elements. Both the nerve-cord and the nerves are, on the other hand, separated from the heart-muscle by the basement membrane. This allows a complete removal of the nervous complex without any injury to the heart-muscle.

The connections between this cardiac nervous complex and the brain and the abdominal ganglia are shown in Figs. 24 and 25. These connections have been carefully worked out by Patten and Redenbaugh, but these investigators failed to find any connection between the hæmal nerves given off from the posterior end of the brain (7, 8) and the nerve-cord on the heart. These

nerves do make such a connection, as I have represented in Fig. 24. This is also borne out by the physiological experiments.

From the dorsal side of the brain corresponding to each pair of nerves to the ambulatory appendages is given off a pair of nerves which take a dorsal direction to innervate the integument, the viscera, and dorsal musculatures. These nerves are evidently homologous to the nerves occupying a similar relation to the thoracic ganglion in *Palinurus* (Fig. *22*, 1–6). The anterior pair of nerves from each abdominal ganglion takes a similar course and make connections similar to those from the dorsal side of the brain. As these nerves go to innervate organs lying dorsal or hæmal to the level of the central nervous system, Patten and Redenbaugh call them hæmal nerves in contradistinction to the nerves to the ambulatory appendages and the gills which are designated as neural nerves.

The hæmal nerves from the abdominal chain of ganglia (Fig. *25*, 9–13) takes a dorsal and posterior direction and after giving off their fibers to the intestine and the integument, penetrate the pericardial cavity, each sending a small fillament along the dorsal wall of the pericardial cavity to unite with the nerve-cord on the heart approximately opposite the fourth to the eight pairs of ostia. These cardiac branches are, with the exception of those from the ninth and the tenth nerves, so tiny that they are not readily made out in the living tissue. Prior to the entrance of these nerves into the dorsal pericardium to connect with the nerve-cord each nerve sends a communicating branch to the nerve which runs parallel to the heart in either angle of the pericardial cavity. These nerves are designated as pericardial nerves by Patten and Redenbaugh.

Of the hæmal nerves given off from the brain the only ones that I was able to trace to the heart are the last two pairs (Fig. *25*, 7, 8). The cardiac branches of these nerves unite in one common trunk before reaching the pericardial cavity. The main branches of this large nerve go to make up the pericardial nerves and to innervate the large inter-tergal muscle, which lies dorsal and lateral to the heart in this region. The branches that pass to the epidermis in the median line connect with the nerve-cord on the heart in the manner shown in Fig. 24. There is considerable individual variations as to the exact place of con-

nection with the nerve-cord, in some specimens there appears to be connections only at the level of the second pair of ostia, in others the connection is made in the middle of the third segment, while in some the main, if not the only, connection is the one just behind the third pair of ostia.

It is rather difficult to homologize the cardiac innervation of the crustaceans with that of *Limulus*. In the crustacean heart the ganglion cells are not congregated in a single ganglion on the surface of the heart, but scattered throughout the muscle. There appears, however, to be this homology that in the crustacean heart the ganglion cells are massed particularly at the posterior end of the dorsal wall of the heart. In *Palinurus* the cardiac nerves take their origin from the anterior end of the thoracic ganglion, the abdominal ganglia not making any connections with the heart; in *Limulus* the cardiac nerves take their origin from what actually corresponds to the thoracic ganglion and from the abdominal ganglia as well. This difference is probably due to the fact that the *Limulus* heart retains its primitive elongated character while the crustacean heart is very much shortened and confined to a small space in the cephalothorax.

Regarding the innervation of the heart in insects we have the observations of Müller and Brandt (quoted by Kolbe, 1893) that the heart and the aorta is innervated from the second pair of œsophageal ganglia. This cardiac nerve would thus seem to correspond to the "cardiac nerve of Lemoine" in the crustaceans, which, as we have already pointed out, appears not to have any basis in fact. To my knowledge no connection between the abdominal ganglia and the heart analogous to that in *Limulus* has been traced in the insects.

Dogiel (1877) has described ganglion cells on the heart or in close proximity of the heart of the larva of *Corethra plumicornis*. According to Lang (1900) a nerve or nerve-cord on the dorsomedian side of the heart similar to that in *Limulus* has been described in some of the myriapods (*Peripatus*, *Jules*).

11. *The Tunicates.* — Despite numerous researches with the view of finding nerve cells and nerve fibers in the heart of the tunicates the results have been, until a recent date, uniformly

negative; neither nerve cells nor nerves could be found. The uniform negative results were generally taken to indicate that nervous elements are not present in the heart of this interesting group of animals. But Hunter (1902) has recently found nerves and nerve cells in the heart of *Molgula manhattensis*. The nerve cells, mostly of the bipolar type, are most abundant at either end of the heart, situated on the surface of the muscular walls under the pericardium. The same observer has later (1903) found evidence to the effect that the cluster of nerve cells or ganglia at either end of the heart are connected by nerve fibres with the central nervous system.

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Explanation of Figures.

Plates IV–VIII.

Abbreviations.

A, anterior artery.
AC, abdominal commissures.
AOS, aortic sinus.
AU, auricle.
B, brain.
BG, branchial ganglion.
CC, cerebral commissure.
CG, cerebral ganglion.
CPC, cerebro-pedal commissure.
CPG, cerebro-pleural ganglion.
CVC, cephalic vena cava.
CVCO, cerebro-visceral commissure.
G, gastric ganglion.
GI, genital ganglion.
GN, genital nerve.
GV, gill ventricle.
H, heart.
HG, hind gut.
IC, intestino-visceral commissure.
K, kidney.
LA, lateral arteries.
LN, lateral nerves.
LVG, left visceral ganglion.
LVN, left visceral nerve.
MNC, dorso-median nerve-cord.
OC, œsophageal commissure.
OE, œsophageal opening.
OER, œsophageal nerve-cord.
OS, ostia.
PA, posterior aorta.
PAM, posterior adductor muscle.
PC, pedal cord.
PCN, pericardial nerves.
PG, pedal ganglion.
PLG, plural ganglion.
PIC, pleuro-intestinal commissure.
PLC, pleuro-visceral cord.
PN, pallial nerve.
PVG, pleuro-visceral ganglion.
PS, posterior sinus.
R, rectum.
RCN, recurrent nerve.
RV, renal vein.
RVG, right visceral ganglion.
RVN, right visceral nerve.
SA, sternal artery.
SL, suspensory ligaments.
SOG, supraœsophageal ganglion.
SG, subintestinal ganglion.
SUG, supraintestinal ganglion.
SV, systemic ventricle.
THG, thoracic ganglion.
V, ventricle.
VC, visceral commissure.
VG, visceral ganglion.
VN, visceral nerve.

Explanation to Plate IV.

Fig. 1. Mytilus californianus. Dorsal view. 1, posterior mantle nerve; 2, branchial nerve; 3, nerve to posterior adductor muscle; 4, nerves to dorsal body wall and pericardium; 5, nerves to base of auricles; 6, nerves to body wall and pericardium; 7, nerves passing posteriorly in the dorsal body wall; 8, 9, 10, ganglia on nerves 4; 11, nerves from the cerebro-visceral commissure to the kidneys; 12, nerves from the cerebro-visceral commissures to the adductor muscles of the foot and the byssus.

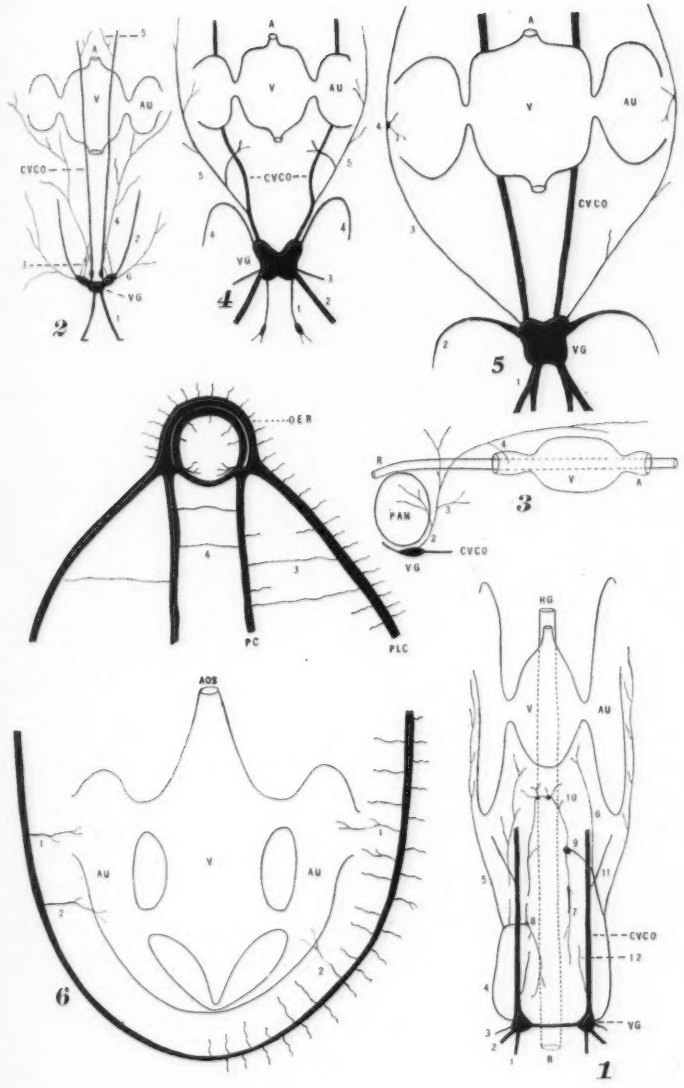
Fig. 2. Mya arenaria. Ventral view. 1, nerve to mantle and siphon; 2, branchial nerve; 3, nerve plexus on the ventral surface of the kidney between the cerebro-visceral commissures and the visceral ganglion; 4, nerves to kidney (and heart); 5, nerves from the cerebro-visceral commissure to the reproductive glands; 6, nerves to kidney.

Fig. 3. Mya arenaria. Lateral view. 1, nerve to mantle and siphon; 2, nerve to posterior adductor muscle, dorsal body wall and pericardium; 3, branch to kidney; 4, branch to posterior aorta and hind gut.

Fig. 4. Tapes staminea. Ventral view. 1, posterior mantle nerves; 2, siphonal nerves; 3, nerves to posterior adductor muscle; 4, nerves to osphradium and gill; 5, reno-cardiac nerves.

Fig. 5. Platydon cancellatus. Ventral view. 1, nerves to siphon and mantle; 2, nerves to osphradium and gill; 3, nerves to renal organ and heart; 4, ganglia at the base of the auricles.

Fig. 6. Cryptochiton stelleri. Ventral view. 1, nerves from pleuro-visceral cords to auricles; 2, nerves that can be followed to the point of attachment of the auricles to the body wall; 3, 4, connectives between the nerve cords.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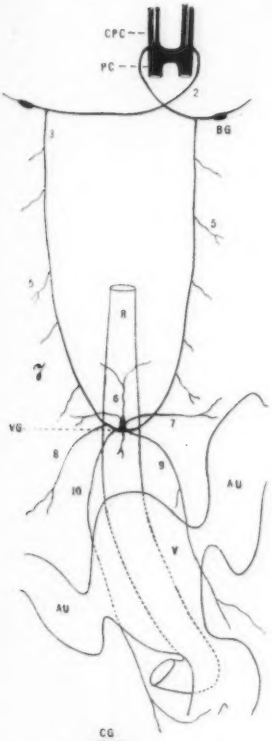
Explanation of Plate V.

Fig. 7. Haliotis cracherodii. Dorsal view. 1, 2, pleuro-visceral commissure; 3, 4, nerves to the visceral ganglia; 5, nerves to floor of pallial cavity and efferent gill sinus; 6, nerve to rectum; 7, 8, nerves to base of auricles; 9, nerve to dorsal pericardium; 10, nerve to ventral wall of paricardium and aorta.

Fig. 8. Lucapina crenulata. Dorsal view. 2, nerve to afferent gill sinus; 3, 4, 5, nerves to the wall of paricardial cavity; 6, nerve to rectum; 7, nerves to renal organs; 8, nerve to mantle fissure; 9, nerve to efferent gill sinus; 10, nerve to osphradium and gill.

Fig. 9. Natica lewissii. Dorsal view. 1, nerve to dorsum; 2, nerve to floor of pallial cavity; 3, nerve to osphradium; 4, nerve to gill; 5, commissure between branchial and left visceral ganglia; 6, nerve to pericardium, heart and kidney; 7, ganglia on commissure; 8, genital nerve; 9, nerve to paricardium, heart and kidney.

Fig. 10. Sycotypus canaliculatus. Dorsal view. 1, nerve to floor of pallial cavity; 2, nerve to pericardium and auricle; 3, nerve to ventricular ganglion; 4, nerve to liver and genital organs; 5, nerve to ventricular ganglion; 7, renal nerve; 8, ganglion on ventriculo-aortic junction; 9, ventricular nerves; 10, pleuro-branchial commissure; 11, nerve to hind-gut.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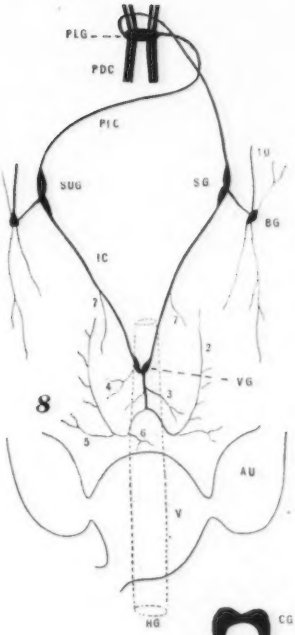
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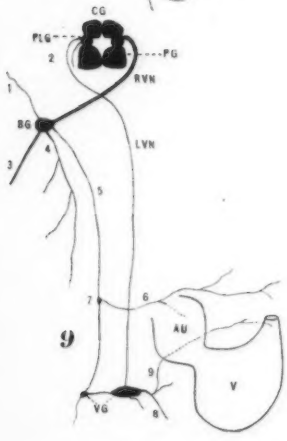
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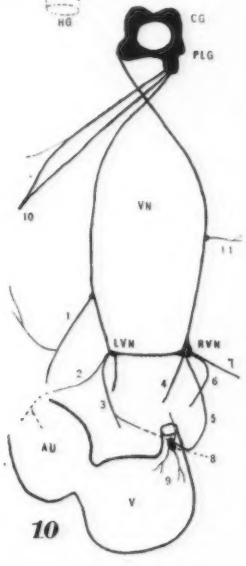
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Explanation of Plate VI.

Fig. 11. Aplysia californica. Dorsal view. 1, nerve to osphradium ; 2, branch to roof of gill chamber ; 3, nerve to viscera ; 4, nerve to gill ; 5, nerve to dorsum of the anal region ; 6, nerve to liver, ventricle and reproductive glands ; 7, nerve to pericardium, kidney and auricle ; 8, branches to kidney ; 9, branch to auricle ; 10, branch to liver ; 11, branch to the reproductive gland ; 12, branch to aortic sinus ; 13, nerve connecting right pedal and visceral ganglia.

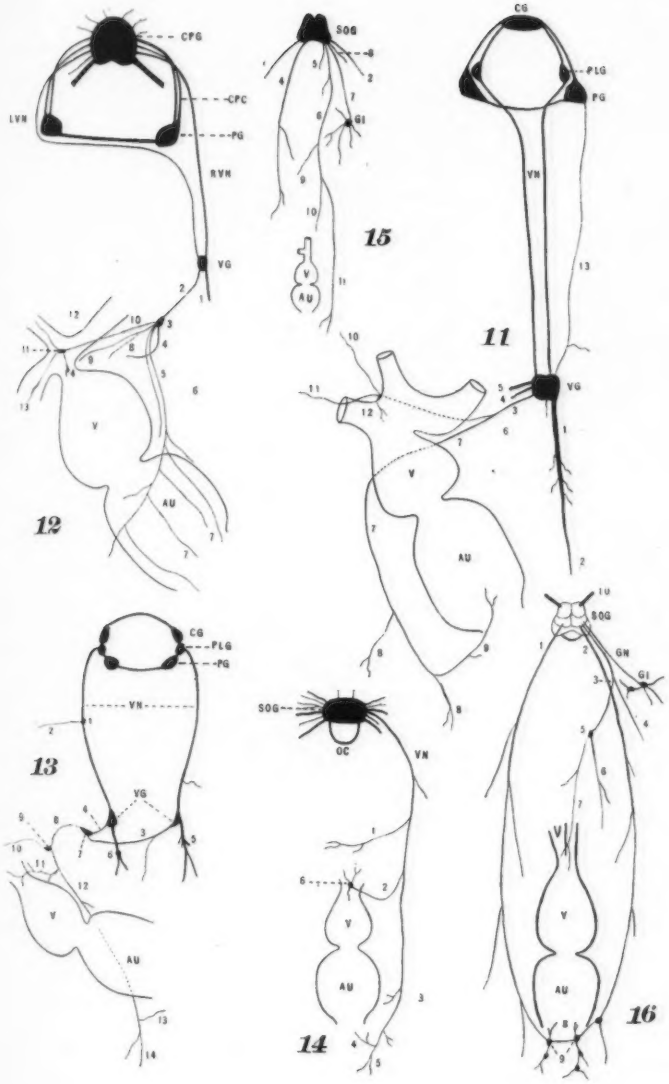
Fig. 12. Pleurobranchæa californica. Dorsal view. 1, nerve to osphradium and gill ; 2, nerve to viscera ; 3, accessory visceral ganglion ; 4, genital nerve ; 5, nerve to pericardium and auricle ; 6, 8, branches to pericardium ; 7, nerves in the walls of the auricle ; 9, branch pericardium and aortic sinus ; 10, branch to aortic sinus ; 11, ganglion on aortic sinus ; 12, branch to the lateral artery ; 13, branch to posterior artery ; 14, branch to aortic sinus, probably entering the ventricle.

Fig. 13. Bulla globosa. Dorsal view. 1, ganglia on left visceral nerve; 2, nerve to dorsum of neck; 3, commissure between the right and the accessory visceral ganglia ; 4, commissure between the left and the accessory visceral ganglia ; 5, nerve to osphradium, gill and roof of gill chamber ; 6, nerve to inferior pallium and dorsum ; 7, accessory visceral ganglion ; 8, nerve to viscera ; 9, ganglion on visceral nerve ; 10, genital nerve; 11, branch to aortic sinus and aorta ; 12, nerve to pericardium, kidney and auricle ; 13, branch to auricle ; 14, branch to kidney.

Fig. 14. Montereina nobilis. Dorsal view. 1, branch of visceral nerve passing ventral to the hind gut ; 2, branch to ganglion on the aortic sinus ; 3, branch to pericardium ; 4, branch to base of auricle ; 5, branch to gill ; 6, ganglion on the aortic sinus.

Fig. 15. Triopha carpenteri. Dorsal view. 1, 2, nerves to the mantle ; 4, left visceral nerve ; 6, right visceral nerve ; 7, nerve to genital ganglion ; 10, branch to anterior aorta ; 11, branch to gill and auricle.

Fig. 16. Triopha grandis. Dorsal view. 1 and 2, nerves to dorsum and gill ; 3, reno-cardiac nerve ; 5, ganglion on the reno-cardiac nerve ; 7, nerve aorta and ventricle ; 8, auricular nerves ; 9, ganglia at the base of the gill ; 10, optic nerve.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Explanation of Plate VII.

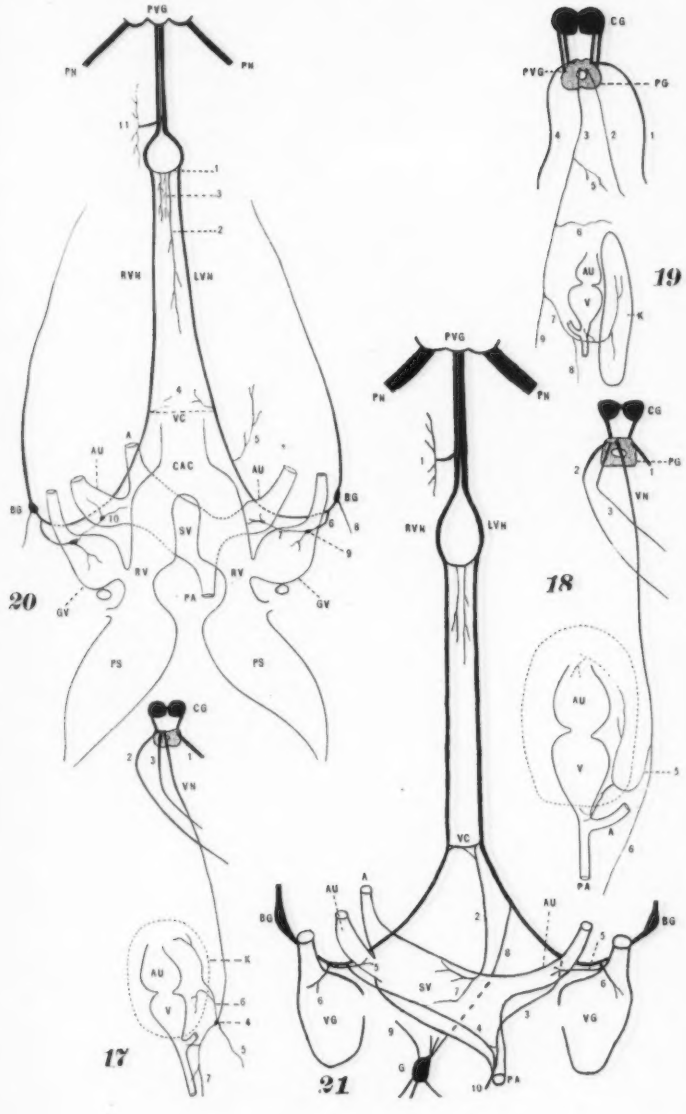
Fig. 17. Limax maximus. Dorsal view. 1, 2, 3, nerves to mantle and pallial cavity; 4, visceral ganglion; 5, genital nerve; 6, reno-cardiac nerve; 7, nerve to liver and intestine.

Fig. 18. Ariolimax columbianus. Dorsal view. 1, 2, 3, nerves to mantle and pallial cavity; 5, reno-cardiac nerve; 6, nerve to intestine and liver.

Fig. 19. Helix (Arionta) dupetitouarsi. Dorsal view. 1, 4, nerves to mantle; 2, nerve to pallial cavity; 3, visceral nerve; 5, branch to genital organs; 6, branch to lung; 7, reno-cardiac nerve.

Fig. 20. Loligo pealii. Ventral view. 1, commissure between the visceral nerves ventral to the vena cava; 2, nerve to the viscero-pericardial envelop; 3, nerves to rectum and duct of the ink gland; 4, nerves to ink gland; 5, nerve to penis; 6, cardiac nerve; 7, auricular nerves; 8, nerves to adductor muscles on the gills; 9, ganglia on gill ventricles; 10, ganglia on auricles; 11, nerves to vena cava.

Fig. 21. Ommastrephes illecebrosa. Ventral view. 1, nerve to vena cava; 2, nerve to ventricle; 3 and 4, nerves to genitalia and osphradium; 5, nerves to auricles; 6, nerves to the gill ventricles; 7, anastomosing branch between nerve 4 and the ventricular nerve; 8, nerve connecting the left visceral nerve with the gastric ganglion; 9, commissure between the brain and the gastric ganglion.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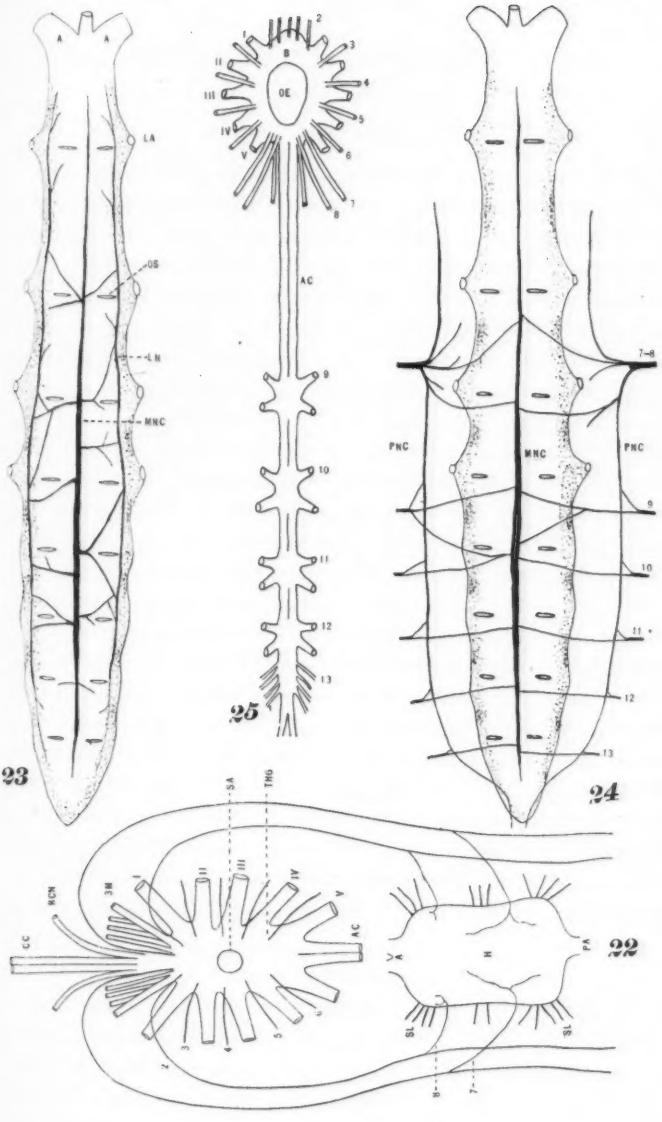
Explanation of Plate VIII.

Fig. 22. Palinurus sp. Dorsal view, heart displaced posteriorly. I–V, nerves to corresponding ambulatory appendages; 3*m*, nerve to third maxilliped; 1, 2, nerves to adductor muscles and heart; 3–6, nerves ramifying in the arterial plexus on the adductor muscles; 7, 8, cardiac nerves.

Fig. 23. Heart and heart-nerves of Limulus polyphemus. Dorsal view. *a*, anterior artery; *la*, lateral arteries; *ln*, lateral nerves; *mnc*, median nerve-cord; *os*, ostia.

Fig. 24. Heart of Limulus. Dorsal view. Showing the connection of the ventral with the cardiac nervous system. *mnc*, dorso-median nerve-cord on the heart; *pn*, pericardial nerves; 7, 8, branches from the two posterior hæmal nerves from the brain; 9–13, branches from the hæmal nerves of the abdominal ganglia.

Fig. 25. Brain and abdominal ganglia of Limulus. Dorsal view. I–V, nerves to corresponding ambulatory appendages; 2–8, hæmal nerves from the brain. The nerves 7 and 8 carry the inhibitory fibers to the heart. 9–13, hæmal nerves from the thoracic ganglia, carrying accelerator fibers to the heart.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THE SPERMATOGENESIS OF THE SPIDER.

With Plates IX. and X.

LOUISE BAIRD WALLACE.

About four years ago, when the present work was undertaken, several investigators had described a peculiar chromosome in insect spermatogenesis, but outside of the insects it had not then been reported and it was a matter of considerable interest to discover whether or not it obtained generally in the maturation of the germ cells of the Arthropoda. Since Dr. Montgomery had failed to find it in *Peripatus*, he suggested to me the advisability of trying some higher form. A study of the reproductive organs of *Limulus* was first attempted but these proved unfavorable on account of the minuteness of the cells. The testes of the spider were then examined and were found to be promising material for, as an offset to the rather large number of chromosomes characteristic of the Arachnida, the germ cells are of fairly good size and the peculiar chromosome forms a conspicuous structure of the cell even to a casual observer.

For various reasons the common tube-weaving spider, *Agalena nævia*, has been taken as the basis for work, but a comparative study of a few representatives from the Drassidæ, Attidæ, Lycosidæ, Theridiidæ and Epeiridæ was found of great value in the interpretation of some points. After the publication of a brief preliminary in the fall of 1900, the problem was laid aside until the fall of 1903, when it was resumed at the University of Pennsylvania uuder the direction of Dr. E. G. Conklin. It gives me pleasure to cordially express my indebtedness to Dr. Conklin for his ever ready encouragement and helpful criticism throughout the year.

Method.

By almost common consent the strong solutions of Flemming's chromo-aceto-osmic mixture and Hermann's platino-aceto-osmic mixture have been chosen as giving the finest fixation for work in spermatogenesis and while I have used a number of different fluids, I can add my testimony to the superiority of the

preparations when the tissues have been treated with one or the other of these two solutions. In order to check as far as possible any changes in the cells which might take place after the death of the animal the spiders were decapitated and the testes rapidly dissected out while the visceral mass was immersed in the fixing fluid. The paired, slightly convoluted tubular organs lie embedded in the liver and contain no pigment so that in immature spiders it is not always an easy matter to distinguish them from the spinning glands which lie ventral to them. As in many other forms, a cross section of the spider testis shows a more or less complete series of developmental stages, with the spermatogonia at the periphery and the cells gradually increasing in maturity toward the lumen. In the breeding season the lumen of the tube is found to be full of mature spermatozoa. Ripe spermatozoa were also taken from the pedipals and studied in smear preparations, sometimes stained *intra vitam* and sometimes fixed, before staining, by heating at the boiling point. Of staining methods, Heidenhain's iron-hæmatoxylin and Hermann's triple stain yielded the finest results.

The Spermatogonia.

The only comparatively recent work upon spider spermatogenesis of which I have any knowledge is that of J. Wagner. Unfortuately his paper is not accessible to me, but from his preliminary report and from several full reviews, I have been able to learn enough of his results to know that they differ widely from mine. He mentions the fact that the spermatogonia are larger than the spermatocytes even at the close of the growth period of the latter. This is true of the spermatogonia of the last generation, but in the early generations the cells are smaller. During the rest stage, when the chromosomes become granular and the chromatin granules are distributed along the linin reticulum, two chromosomes are conspicuous because they take no part in this disintegration (Pl. I., Fig. 1). From this point to the formation of the spermatozoön these two chromosomes can be identified by their shape, by their peripheral position and by the fact that they show a strong affinity for safranin, in the Hermann's staining method, while the other chromosomes take the gentian

violet excepting in the metaphase and anaphase, when they also take the safranin. By some authors this is supposed to be due to the presence of a greater amount of nucleinic acid at the height of the mitotic division and since the two peculiar chromosomes in the spider show this reaction constantly throughout the maturation processes it seems possible that they are more richly supplied with nucleinic acid than the other chromosomes. This is of special interest when we recall that the nuclei of spermatozoa contain a maximum of phosphorus.

The rod-shaped chromosomes are so numerous that it is difficult to make an accurate estimate of their number but by repeatedly counting those found in a cross section of the equatorial plate I believe the number to be forty (Pl. I., Fig. 2). In such a polar view of the equatorial plate two of the forty chromosomes are found in a peripheral position, thus reducing the number of other chromosomes to thirty-eight. The ordinary mitotic division occurs in which forty daughter chromosomes are carried to each pole, so it is puzzling to know why Wagner should say "Die Kerne der spermatogonien theilen sich nicht nach dem gewöhnlichen schema der Karyokinese aber auch nicht amitotisch." During mitosis the two peripheral chromosomes split longitudinally and are equally distributed to the two poles, just like the other chromosomes (Pl. I., Figs. 3, 4 and 5). During the telophase when the daughter chromosomes become granular and begin to spread out on the linin reticulum — even at the beginning of this disintegration — two are conspicuous because they remain unchanged and in the rest stage these two rods are sharply defined as they lie in the chromatin reticulum. Usually they lie side by side but are sometimes found at a short distance from one another and I am inclined to think that in the latter case they are slightly dislodged by the microtome knife and so become separated (Pl. I., Figs. 6, 7 and 8.)

A word might be said here in regard to the name of the peculiar chromosomes. In a recent paper ('04) Montgomery has suggested the general term heterochromosome to include the nuclear elements which have been described under various names, and he then divides them into two groups, according to their origin, as follows: (1) "The accessory chromosome"

(McClung), unpaired in the spermatogonia, as found in the Orthoptera. This would include the "chromosome spécial," described by de Sinéty; (2) the "chromatin nucleoli" (Montgomery), paired in the spermatogonia as found in *Euchistus*. This would include the "small chromosome" of Anasa, described by Paulmier. Such a classification does not apply in the spider, where the heterochromosomes are similar in appearance and behavior to those described by McClung as accessory chromosomes, but differ from them in arising as a double element. Sutton's work on *Brachystola* plainly shows the accessory chromosome to arise as a single element in the spermatogonia. The heterochromosomes of the spider, so far as their double origin is concerned, resemble the chromatin nucleoli of *Euchistus* and the "small chromosomes" of *Anasa*, but are otherwise different from them. In view of these facts, McClung's term is employed in this paper, and as the accessory chromosomes never fuse together, but only lie in close contact, the term will be used in the plural.

Primary and Secondary Spermatocytes.

Early in the prophase of the primary spermatocyte the chromatin is finely distributed on a delicate linin reticulum and the accessory chromosomes retain their individuality (Pl. I., Fig. 9). This stage I believe to be comparable to the synapsis, for at this time, or possibly earlier, the pseudo-reduction occurs. In none of my best preparations do I find a massing of the chromatin at one side of the nuclear cavity. Such a massing is found in poorly fixed material, but even then it occurs only in the later spireme stages. In his paper on *Peripatus* Montgomery has asserted that in synapsis is accomplished an end-to-end union, in pairs, of entire chromosomes, and that this numerical reduction occurs during the retrogressive stages of the telophase of the last spermatogonic division. Blackman, in his work upon Myriapods, in discussing this point, adds: "It can be stated with the greatest certainty that pseudo-reduction occurs during the telophase of the last spermatogonium and is completed before the reconstruction of the nuclear membrane." From the synapsis stage, represented in Fig. 9, arises the spireme. Like other workers, I find it impossible to state positively that it is segmented from the first

but I think such to be the case, and also that the nineteen loops are connected by a band of linin only. These loops grow thicker and show a longitudinal split gradually increasing in width, while the nucleus becomes more swollen with nuclear sap (Pl. I., Figs. 10–13). At this time a definite polarity can be noted, the blind ends of the loops being directed away from that portion of the cell which contains the greater mass of cytoplasm and the centrosome. The accessory chromosomes lie in the embrace of the free ends of a loop and near the centrosome. In testes nearing maturity, the majority of the cells are in this stage. This is followed by a rather rapid shortening of the loops when they draw down toward what Montgomery has called the distal pole (Pl. I., Figs. 14, 15). In his description of this stage Wagner says: "Der Linin faden (resp. die Reihen der chromatin-körner) bildet Schleifen, die alle gleich lang sind und die gleich Richtung haben: in dieser Weise theilt sich der Linin faden in Stücke von gleichen Länge. Gleichzeitig bildet sich der Nucleolus." While I agree with him in regard to the same general direction of the loops I do not agree with him in his statement that they are equal in length. At this stage the difference in the size of the loops can be plainly seen. This condition favors Montgomery's view of the end-to-end conjugation of chromosomes of like size during synapsis and which has been confirmed by Sutton in his work on *Brachystola*. It seems highly probable, in the light of recent research, that Montgomery's theory in regard to the pairing of paternal and maternal chromosomes during synapsis is the true explanation of the numerical reduction occurring at this time.

In the prophase of the primary spermatocytes, the bend of the loops becomes more acute, while the arms shorten and thicken and the now V-shaped chromosomes, varying in size, are scattered through the nuclear cavity. In doubly-stained material the accessory chromosomes appear as two red rods lying side by side among the violet V-shaped chromosomes. The latter now split from apex to base, opening out to form double V's and, sometimes after, sometimes during this process are drawn into the equator of the first maturation spindle (Pl. I., Figs. 16–20). In the metaphase the accessory chromosomes always take a per-

ipheral position, are connected by linin fibers to one pole only, and are drawn to this pole before the daughter V-shaped chromosomes are more than half way to their destination (Pl. I., Figs. 23, 24).

According to Wagner — "bei der ersten spermatocytentheilung theilt sich der Nucleolus entweder in der Ebene der Äquatorialplatte mit den Chromosomen zusammen oder auszerhalb denselben neben einem der spindelpole." In the latter case he believes it to be cast out into the cytoplasm. Here, as elsewhere, what he considers to be a "Nucleolus" is without doubt the pair of accessory chromosomes which often lie closely apposed to one another and arrive at one pole before the other chromosomes. Their eccentricity of position might mislead one into thinking that they are being thrown out of the nucleus.

In every case where a section is found cutting through the equatorial plate of the primary spermatocytic monaster transversely, cross-sections are found of the nineteen double V-shaped chromosomes and frequently are found also the accessory chromosomes which appear larger because of their oblique position in relation to the spindle axis. Their nearness to the cell-wall is here quite clearly demonstrated (Pl. I., Figs. 21, 22). During the telophase the nineteen V-shaped chromosomes enter into a partial rest, becoming granular but not forming a reticulum, while the accessory chromosomes in one of every two daughter nuclei stand out in a striking manner as two densely stained rods against the granular background and in sections stained with Hermann's method, their affinity for the safranin at a time when the other chromosomes take the gentian violet, makes them still more prominent (Pl. I., Fig. 25).

The nuclear membrane now forms, one nucleus containing a pair of accessory chromosomes, the other none (Pl. I., Fig. 26), and the V-shaped daughter chromosomes, attached by their apices are quickly drawn into the equator of the spindle for the second maturation division (Pl. I., Fig. 27). Frequently the cell body of the primary spermatocyte does not undergo division until toward the close of the telophase of the second maturation division.

SEQUENCE OF REDUCTION AND EQUATIONAL DIVISIONS.

Most authors now grant that one transverse and one longitudinal division obtain in the maturation divisions of the germ cells, but there is still a lack of agreement in regard to the sequence of these two divisions. There has been a decided majority in favor of the view that the longitudinal division occurs first. Montgomery has emphasized the importance of determining the origin of the chromosomes in order to get at the truth of this matter and he claims to have established the fact that "the heterotypic mitosis, the first maturation mitosis, is not an equation division but separates entire univalent chromosomes, while the second maturation mitosis is equational." This conclusion is supported by the work of Korschelt on the ovogenesis of an annelid, by the work of Henking and Paulmier on Hemiptera, fo Miss Nichols on isopods and by several others. There is the

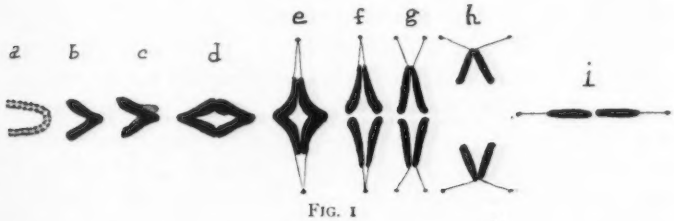

FIG. 1

possibility that uniformity in regard to the sequence of the divisions does not exist since in either case the ultimate result is the same, but if there is uniformity the spider spermatogenesis seems to me an especially good field for determining this point and it shows strong grounds for believing that the first division is a reduction division. The process is as follows: When pseudo-reduction occurs in synapsis, giving rise to nineteen loops of different lengths, there is at first no split visible in the thread of the loop. When the split does appear it becomes steadily more noticeable during the growth period and is then obscured in the condensation of the chromatin to form the thickened V-shaped chromosomes (text figure, *a* and *b*). In the prophase of the primary spermatocyte, the V's split from apex to base, parting along the line, I maintain, of the original, longitudinal split of

the loops in the spireme (*a* and *c*), and then open out into double V's (*d*). In the metaphase these double V's are so placed at the equator of the spindle that the longitudidal split is parallel to the spindle axis. This can be determined with certainty since often the splitting occurs after the chromosomes have taken up their position in the equatorial plane and can be seen at different stages of the process, the line of the split being always parallel to the spindle axis (Pl. I., Figs. 18, 19, and text figure, *e*).

Double spindle fibers connect the ends of the double V-shaped chromosomes with the centrosomes. To express it in another way, — after a V-shaped chromosome has become a double V by a split passing longitudinally along each arm, each half of each arm is connected by one linin fiber with the centrosome, but since the distal ends of every such pair are in close juxtaposition, we have the appearance of double spindle fibers passing from them to the poles. The first maturation division then takes place through what corresponds to the apex of the original V's and is a transverse or reduction division (*f*). When the centrosomes divide to form the daughter centrosomes for the second maturation spindle, one of each pair of linin fibers remains attached to one of the daughter centrosomes. When the latter have moved in opposite directions and have taken up their positions at right angles to the position of the mother centrosome (thus becoming the poles of the second maturation spindle) the V-shaped daughter chromosomes, formed from the longitudinal splitting of a single arm, are left attached by their apices in the equator of the spindle. The space between the arms of these single V's corresponds to the space between the halves of the longitudinally split spireme and, therefore, when the arms of the V are drawn to opposite poles, we plainly have an equational division (*g*, *h*, *i*).

To sum up: The first division occurs at a point corresponding to the bend of the loop in the spireme while the second occurs along the line of the original, longitudinal split of the spireme. Such an interpretation must surely be the true one if it be granted that the loops arise in the synapsis by a conjugation, end to end, of like chromosomes. And, even if the loops are supposed to originate by the spireme simply breaking up into the reduced number of chromosomes, still the sequence of the divisions would be as given above.

Significance of the Accessory Chromosome.

Considerable attention has been paid to the distribution of accessory chromosomes to the spermatids because opinions have differed in regard to it. Nothing could be clearer than the fact that, in the spider, during the first maturation mitosis, the two accessory chromosomes pass bodily over into one of the two daughter cells. In the second maturation mitosis the question is far more difficult to answer, partly because but one half of the cells contain the accessory chromosomes anyhow and partly because the V-shaped chromosomes attached to the equator of the spindle by their apices, cause a branching in the equatorial plate and thus the monaster is a much less clear subject for study than is found in the first spermatocytic monaster. A number of favorable cases were found, however, in which the two accessory chromosomes pass to but one polè and such cases, together with a careful study of the spermatids, have convinced me that not only in the first division, but also in the second the accessory chromosomes pass to but one pole, and are therefore, distributed to only *one fourth* of the spermatids (Pl. I., Fig. 28). In this respect my results differ from all other workers yet heard from. Henking and Paulmier found the hetero-chromosomes dividing in the first spermatocytic division, but passing undivided to one daughter cell in the second; McClung, Sutton and Blackman hold just the reverse. They find them taking no part in the first but dividing in the second division. In either of the above cases the result would be the same, *i. e.*, one half of the spermatids would contain no heterochromosomes, while the other half would contain them and could be called what Henking has styled "bevorzugten Tochterzellen." Montgomery finds the heterochromosomes dividing like the ordinary chromosomes in both divisions and they are, therefore, present in all of the spermatids, while in the spider they seem to stand aloof in both of the maturation mitoses and are, therefore, distributed to but one fourth of the spermatids.

In consideration of our limited knowledge in regard to the heterochromosome, and of the fact that nothing is known as to what part this peculiar nuclear element plays in fertilization, it is perhaps premature to say anything in regard to its significance.

McClung has suggested that the heterochromosomes might be sex-determinants and he is supported in this view by Sutton, Blackman and others. Paulmier regarded them as degenerating chromatin. Montgomery agreed with him in this and considers that they are "chromosomes that are in the process of disappearance, in the evolution of a higher to a lower chromosomal number." If only one fourth of the spermatids contain the heterochromosomes, as in the spider, they can scarcely be sex-determinants. Neither do they here show the characteristics of degenerating chromatin. That they represent some form of specialized chromatin, I cannot doubt, but that they are specialized for some metabolic function and comparable to nucleoli, as has been suggested by Montgomery, does not seem to me probable in view of the facts. I, therefore, venture to offer a fourth theory in regard to the function of the accessory chromosomes in the spider.

The breeding season of *Agalena nævia* comes late in the summer, the eggs being laid in August and September. While examining preparations made late in September I noticed a great many degenerating cells side by side with the ripe spermatozoa found in abundance in the lumen of the testis and in the ducts (Pl. II., Fig. 49). These cells are without nuclei and many of them are fragmented. My first conclusion was that the presence of so many fragmenting cells was due to the lateness of the season but on examination of sections prepared early in August a few cells undergoing degenerative processes were found even though but few spermatozoa had yet reached maturity. Sections made from the testis of *Pholcus phalangiodes* at the very height of the breeding season, in July, were most convincing. In *Pholcus* the spermatozoa are much more elongated than in any other species of spider examined (Pl. II., Fig. 51), but when mature they curl up as the others do. In the lumen and ducts were found hundreds of spermatozoa thus coiled up but a far greater number of round cells with rounded nuclei in which the linin and chromatin are no longer distinguishable. These cells vary in size — some as large as the germ cells, some very minute and in every way they resemble cells in the process of degeneration (Pl. II., Fig. 50, *d*). Further study showed that as a rule the degeneration does not begin until after the spermatozoa have

been emptied from the cysts into the lumen. The process then goes on very rapidly. Probably the degenerating cells of *Pholcus*, if examined near the close of the breeding season would be found to have lost their nuclei like those of *Agalena nævia*. It was not found possible to make an accurate count of the two kinds of cells in the ducts and hence it was not possible to determine with certainty their relative numbers but it was estimated that there were about three times as many of the degenerating cells as of the normal, ripe spermatozoa. What could be the determining cause? It will be remembered that there are three times as many spermatozoa without the accessory chromosomes as with them, and it occured to me that possibly only one fourth of the spermatids, the "bevorzugten zellen," become functional spermatozoa. Platner and O. Hertwig have shown that the two divisions which form the polar bodies of the egg are homologous to the last two divisions of male germ cells but that while in the female germ cell the products of division are very unequal in size and only one of the four is functional, in the male germ cell they are equal in size and each of the four is functional. If it can be proven that three fourths of the sperm cells are aborted, just as three fourths of the ova are, the parallelism in the spermatogenesis and ovogenesis will be found to be even more complete than has hitherto been supposed. There are at least *a priori* reasons for believing such to be the case and I was still further strengthened in this position when my attention was called to a short paper by Meves in which he describes the finding of "polar bodies" in the testis of the honey bee and other Hymenoptera. "Die Spermatocyten 1. Ordnung bei den genannten Hymenopteren stoszen ebenso wie sonst die Eier bezw. Ovocyten 1. Ordnung nach einander zwei "Richtungskörper" aus; von diesen besitzt jedoch nur der zweite einen Kern, während der erste ausschlieszlich von Cytoplasma gebildet wird . . . . Die ersten Richtungskörpen gehen nach einiger zeit zu Grunde. Die zweiten Richtungskörper dagegen beginnen ebenfalls sich zu Sprermien zu entwickeln, wobei ihre Kerne dieselben Veränderungen wie die Kerne der groszen zellen und zeitlich parallel mit ihnen durmachen-jedoch scheint dieser Entwickelungsprozesz schlieszlich, wenn auch erst sehr spät, zum Stillstand zu kommen und in Degeneration überzugehen."

We might here mention once more that the accessory chromosomes show a staining reaction throughout which the other chromosomes show at the height of mitosis and which some think to be due to the presence of a larger amount of nucleinic acid at that time. Now while staining reactions are not a safe criterion, they may give us at least a rough test of the chemical conditions in a cell, and since the nuclei of spermatozoa have been found to contain a maximum of nucleinic acid, and since the "bevorzugten zellen" in the spider might be richer in nucleinic acid, on account of the presence of the accessory chromosomes, than the remaining three fourths of the male germ cells which are not so favored, we might here see a new significance in the accessory chromosomes.

Spermatids and Spermatozoa.

A study of the spermatids will throw further light upon the subject of the distribution of the accessory chromosomes. In most cases it is evident that when the secondary spermatocytes, which do contain the accessory chromosomes, divide, the latter pass to but one of the resultant spermatids (Plate II., Figs. 29–33). Figs. 34 and 35 show spermatocytes which are not "favored cells." Sometimes each of the two spermatids resulting from the division of a favored spermatocyte seems to contain one accessory chromosome, but I think this should be regarded as an exceptional case (Pl. II., Fig. 37). At a later stage in the formation of the spermatozoön it is impossible to determine which cells contain the accessory chromosomes and which do not. At the beginning of the process of the condensation of the chromatin to form the head, the two accessory chromosomes become closely applied to one another, then appear to fuse together. They still take the safranin stain and form a center into which the chromatin reticulum which takes the violet stain, is gradually drawn until all of the chromatin forms a compact mass stained brilliant red. In the spermatids which lack the accessory chromosomes the chromatin at one point in the reticulum forms a net-knot which now takes the safranin stain and it becomes the center of condensation and so simulates the appearance of the favored cells. At such a stage one might be mislead into think-

ing that every spermatid contains the accessory chromosomes. This stage in *Agalena* is shown in Fig. 38 and in *Epeira*, Figs. 42–44. In all cases the chromatin assumes a crescentic form, the anterior end bends in, the posterior end folds over it and we see the mature spermatozoa each consisting of a crescent-shaped nucleus, covered by a pellicle of cytoplasm and curled up. In this condition they are found in abundance in the lumen of the testis, in the ducts and in the pedipalps (Figs. 39–41).

In Fig. 48 is pictured a spider spermatozoön after Wagner in which he demonstrates a tail. This is of special interest to me because I have not succeeded in finding an organ of locomotion in connection with the spermatozoön. As he also worked upon *Agalena*, I am surprised to find my results so different from his. He claims that "Die Spermatozoen haben auf gewissen Stadien bei allen Species einen typischen Schwanz mit Achsenfaden. Der Achsenfaden bildet sich im Protoplasma der Spermatocyte (resp. Spermatide) zuerst als ein kurzes Stäbchen welchem bisweilen einige Archoplasmakörnchen anliegen. Mit dem Kerne verbindet er sich erst nach dessen Umwandlung in die Chromatinplatte."

"Wo sich Achsenfaden und Chromatinplatte verbinden liegt am Rande der letzeren ein Zähnchen." In striving to get light upon these points I have studied the spermatozoa of about a dozen different species of spiders, have stained them *intra vitam*, have made smear preparations fixed by heating at the boiling point, have studied, with painstaking care, sections fixed and stained in a great variety of ways, and in no case has a tailed spermatozoön been found with the exception of *Pholcus* and even here the elongated portion which might be looked upon as a tail, stains more like a middle piece and its cytoplasmic origin is questionable (Fig. 51). My belief is that the "tail" which Wagner saw was nothing more nor less than the outline of a vesicle which is nearly always in evidence when the spermatid is being transformed into the spermatozoön, and that what he describes as a little tooth is really nothing more than the anterior end of the head bent under, although in this case there should be a rounded bend instead of what he figures (Figs. 46, 47). I would be glad to believe in the existence of a tail and have

earnestly tried to demonstrate it but so far the method of locomotion of the spider spermatozoön remains an unsolved mystery to me. In a future paper I hope to be able to give some light upon this subject as well as upon the rôle played by the accessory chromosomes in fertilization. As my work now leaves it, the functional spermatozoa would contain an uneven number of chromosomes — nineteen ordinary ones and two accessory chromosomes, and it is not clear how the somatic number of forty chromosomes is made up in the cleavage nucleus of the ovum unless it be found that accessory chromosomes are thrown off in the polar bodies, thus leaving the mature ovum with only nineteen chromosomes. These nineteen, added to the twenty-one brought in by the spermatozoön would make the total required; forty chromosomes.

Summary.

1. The spermatogonia contain two accessory chromosomes and thirty-eight other chromosomes.

2. In the primary spermatocytic division the two accessory chromosomes pass over undivided into one of the daughter cells. The reduced number of other chromosomes is nineteen and these divide transversely.

3. In the secondary spermatocytic division, the two accessory chromosomes again pass over undivided into one of the daughter cells. The nineteen other chromosomes divide longitudinally.

4. Only one fourth of the spermatozoa contain the accessory chromosomes.

5. Apparently the remaining three fourths of the spermatozoa degenerate after almost or altogether reaching maturity. In this respect they are regarded as homologous to the polar bodies thrown off by the ovum.

The University of Pennsylvania,
June, 1904.

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Explanation of Figures.

All drawings were made at a magnification of about 2,000 diameters and then reduced one third. All figures were drawn from Agalena unless otherwise specified, and were drawn with the aid of the camera lucida.

Plate IX.

Fig. 1. Spermatogonium in resting stage.

Fig. 2. Pole view of equatorial plate of spermatogonic monaster.

Fig. 3. Spermatogonic monaster.

Figs. 4–5. Anaphase of spermatogonic monaster.

Fig. 6. Daughter cell of last spermatogonial division showing disintegration of the chromosomes.

Figs. 7–8. Resting cells.

Fig. 9. Formation of spireme, "synapsis."

Fig. 10. Completion of spireme.

Figs. 11–13. Coarse spireme with longitudinal split.

Figs. 14–15. Shortening and thickening of loops of segmented spireme.

primary spermatocyte with loops transformed into V-shaped chromosomes.

Fig. 17. Splitting of chromosomes.

Figs. 18–20. Primary spermatocytic monaster.

Figs. 21–22. Sections of equatorial plate.

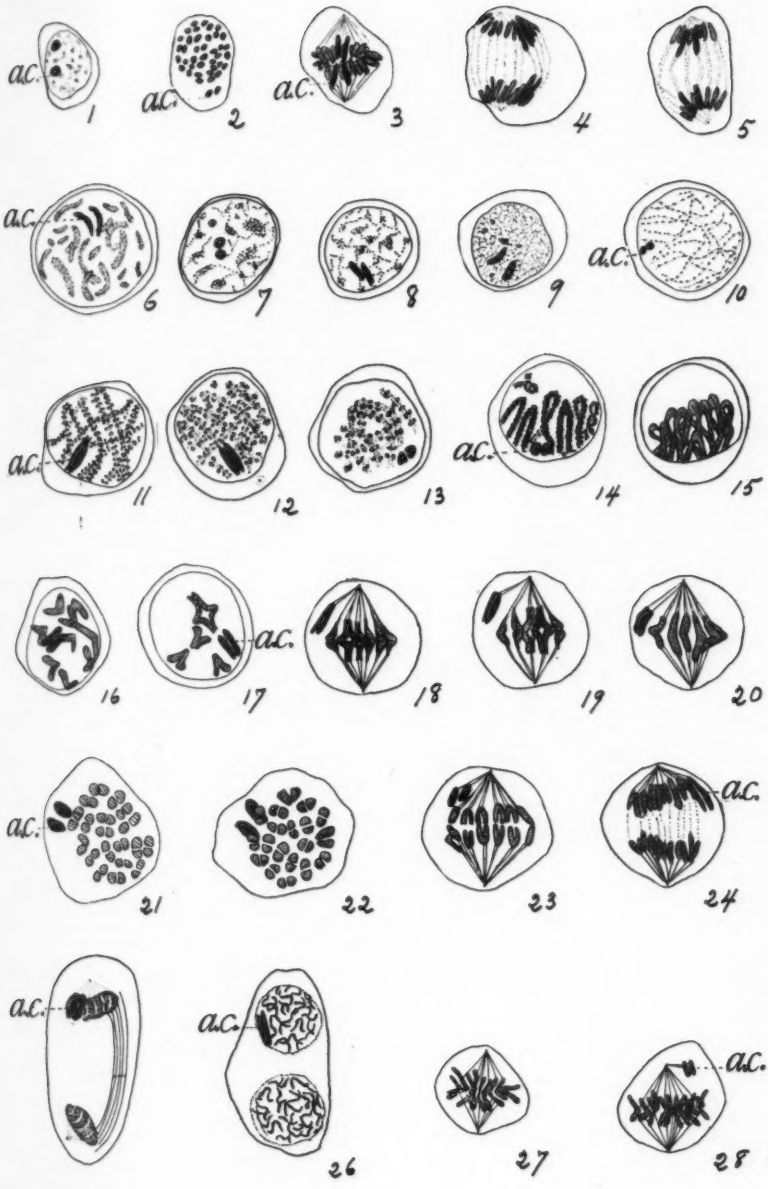
Fig. 23. Primary spermatocytic metaphase.

Fig. 24. Primary spermatocytic anaphase.

Fig. 25. Late anaphase or telophase.

Reconstruction of daughter nuclei.

Figs. 27–28. Secondary spermatocytic monaster.


a.c.
1
a.c.
2
a.c.
3
4
5
a.c.
6
7
8
9
a.c.
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11
12
13
a.c.
14
15
16
17
a.c.
18
19
20
a.c.
21
22
23
24
a.c.
a.c.
26
27
a.c.
28

PLATE X.

FIGS. 29–39. Spermatids.

FIGS. 40, 41, 46, 47. Spermatozoa.

FIGS. 42–45. Spermatids of *Epeira sclopetaria*.

FIG. 48. Spermatozoön of *Agalena*, nach Wagner.

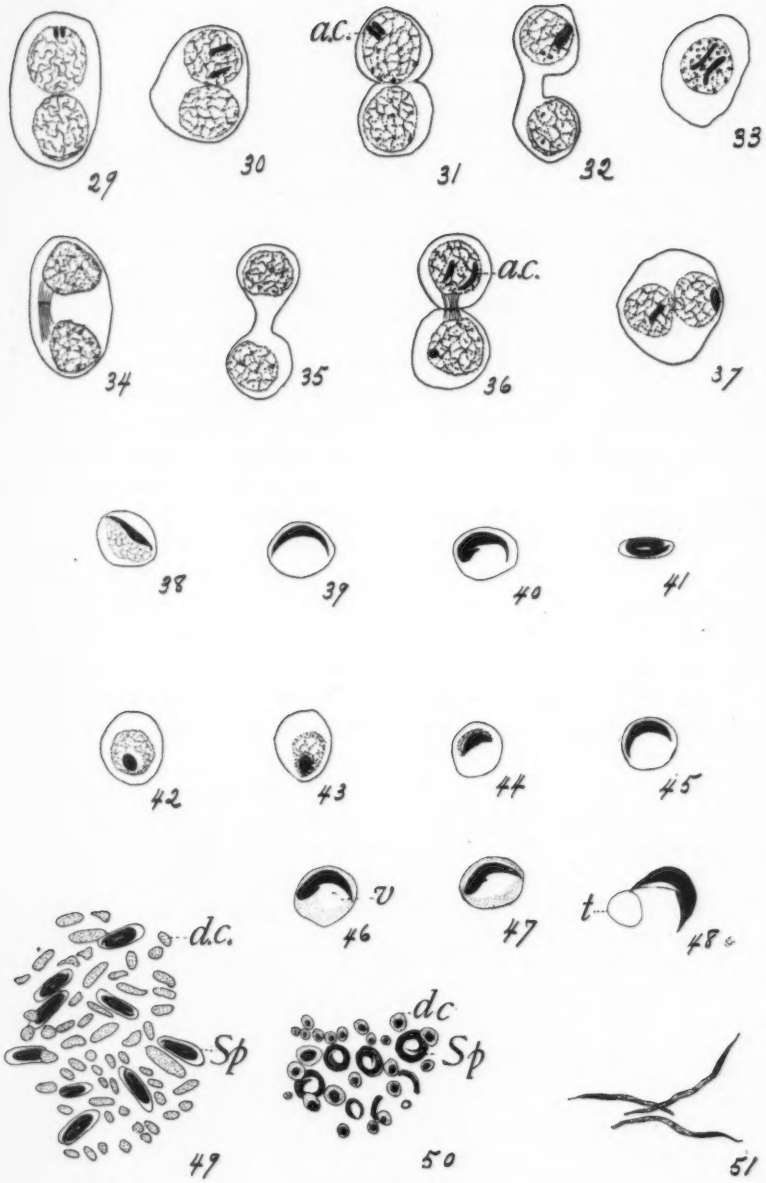
FIG. 49. Normal spermatozoa and degenerating cells. *Agalena*.

FIG. 50. Normal spermatozoa and degenerating cells. *Pholcus*.

FIG. 51. Spermatozoa of *Pholcus phalangiodes*.

ABBREVIATIONS.

a.c., accessory chromosomes; *sp.*, spermatozoön; *d.c.*, degenerating cells; *t.* tail of spermatozoön, nach Wagner; *v*, vesicle.


a.c.
29
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31
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a.c.
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d.c.
Sp
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dc
Sp
50
51

THE FORMATION OF THE INTERIOR CELLS IN THE SEGMENTATION OF THE FROG'S EGG.

MARGARET REED.

Sections through the egg of the frog in the early stages of segmentation show that the cleavage planes extend from the surface inwards forming but one layer of cells. Later cleavage stages, however, show certain cells entirely within the interior of the egg, so that the cells of the dark pole appear to be arranged in two layers about the segmentation cavity.

Former observers supposed this appearance to be caused by a cleavage plane coming in parallel to the surface of the egg (delamination) probably between the 32- and 64-cell period.

Following Professor Morgan's suggestion, I attempted to determine whether a delamination really occurs or whether the interior cells are produced in some other way.

If the delamination occurs between the 32- and 64-cell stages we should expect to find the interval between these cleavage stages, as seen on the surface, longer than that between any two preceding divisions, because somewhere during this time, the cells would have divided by planes, which do not appear on the surface.

In order to test this point, I made a number of observations on the living egg, noticing the times of the surface divisions. I found no marked difference in the length of the interval between any two divisions, but the cleavage took place in a regular rhythm. There was usually a period of about one hour from the first appearance of segmentation in the cells of the dark pole until the next cleavage again began to appear there. This is shown in the following table.

9:25 A. M.	Beginning of	2	-cell	stage	at dark pole.
10:15 "	"	4	"	"	
11:15 "	"	8	"	"	
12:10 P. M.	"	16	"	"	
12:55 "	"	32	"	"	

1:50 P. M.	Beginning of	64	-cell	stage at dark pole.
2:40 "	"	128	"	"
9:25 A. M.		8	"	"
9:30 "	"	16	"	"
10:35 "	"	32	"	"
11:35 "	"	64	"	"
12:25 P. M.	"	128	"	"
1:40 "	"	256	"	"

From this we see that if the delamination should occur between the 32- and 64-cell periods the division must take place at this period twice as fast as any other division. This of itself seemed improbable. I therefore preserved series of eggs taken every five minutes during the interval between the 32- and 128-cell divisions and later sectioned them in order to determine how the interior cells arise.

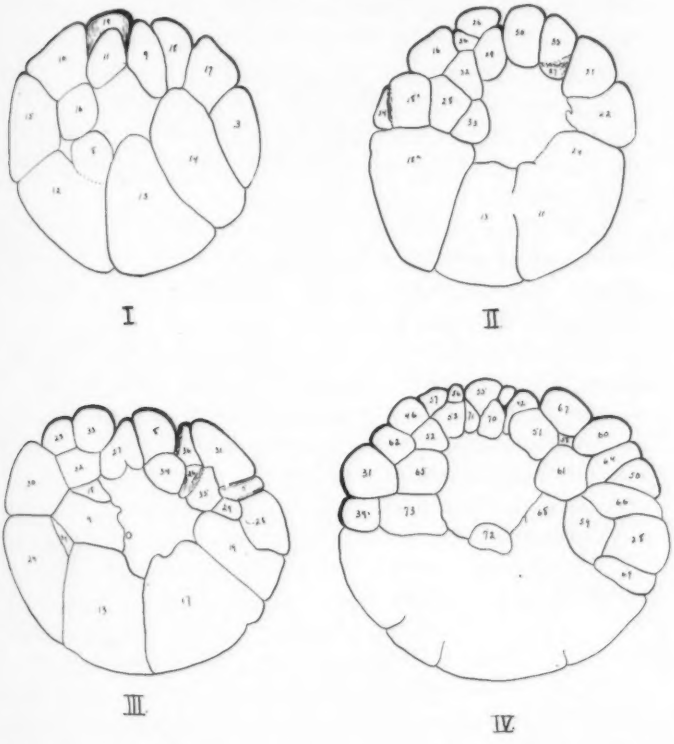
The eggs were cut about 20 μ thick and all the sections drawn with the aid of a camera lucida. Each cell of the egg was numbered and traced through the series of sections, in order to determine not only how many cells were present but also to ascertain how many came to the surface and how many were entirely inside. Two series taken every five minutes between the 32- and 128-cell stages were counted with the following as some of the results:

Supposed Number of Cells.	Actual Number of Cells.	Cells Inside.	Superficial Cells.
32	28	0	28
32	32	0	32
32–64	37	3	34
32–64	45	5	40
32–64	53	6	47
64	64	15	49
64–128	105	26	79

If delamination took place between the 32- and the 64-cell period, sections of an egg at the 64-cell stage should show 64 cells coming to the surface, plus a certain number of cells inside, but this, as the table shows, is not the case.

Sections through the egg as late as the end of the 32-cell stage show that all the cells are divided by cleavage-planes, which appear on the surface. Sections through the eggs of the series between the 32- and 64-cell stage show an increase

in the number of superficial cells[1] as well as of those which are wholly within the egg. At the end of the 64-cell stage there are 64 cells present in the egg, a number, about 12, of which do not appear on the surface. Also the series between the 64- and 128-cell cleavage stages show an increase in the number of cells coming to the surface, as well as the number of cells of the in-



I II III IV

terior, so that there is no cleavage stage between which and the following stage delamination takes place.

Figs. 1, 2, 3 and 4 show sections through the middle of eggs of 28, 53, 64 and 105 cells respectively. A number of cells in

[1] In the cleavage of the frog's egg, the division for the next cleavage stage begins in the cells of the dark pole, while the cells of the light pole are completing the division for the preceeding cleavage stage.

these figures have the appearance of being pushed or crowded into the interior, that is, the portion of the cell which comes to the surface is quite small compared with the size of the cell which lies mainly within the egg.

This appearance of the cells seems to be caused by the division planes coming in more or less obliquely so as to cut off one cell with a small surface area as No. 9, Fig. 1. At the next cleavage, the division of this cell would give rise to one cell entirely within the egg and one coming to the surface. Cell 27, Fig. 3, shows the beginning of such a division while cell 70 or 71, Fig. 10, may have been formed in this manner. The following division of such a cell would then form cells lying entirely within the egg.

So far as I have been able to trace, the yolk cells also show no cutting off of interior cells during the early cleavage stages. Such a cell as 72 in Fig. 4, is but the end of a cell which other sections show coming to the surface.

These observations show that there is no delamination division cutting off a number of cells parallel to the surface but that the first formed cells of the interior are produced by the same division planes as are the cells of the surface and by their subsequent division form the cells of the interior.

COLUMBIA UNIVERSITY,
December 13, 1904.

ON THE CONDITIONS DETERMINING THE DISPOSITION OF THE CHROMATIC FILAMENTS AND CHROMOSOMES IN MITOSIS.

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Regarded from the purely physico-chemical or physiological standpoint protoplasm is essentially a complex aggregate of water and various colloidal and crystalloidal substances of which electrolytes form a chief part. It is also important to note that the *distribution* of the substances composing this aggregate is often, perhaps always, of a highly definite and specific kind; in recognition of this fact a more or less clearly defined "structure" has always been ascribed to protoplasm. Strictly speaking the term "structure" must include in its significance the distribution, grouping and relative positions of *all* the different cell-constituents; ordinarily, however, we do not regard the water and the more readily diffusible substances as distinctively structural elements; in general we mean by such elements those whose position in the complexus remains relatively constant and which give the form to the whole. On this definition we observe that it is primarily the *colloidal* substances of the cell that determine by their arrangement, distribution, and state of aggregation the particular type of structure that is presented; in other words, colloids form the basis of protoplasmic structure.

The reason for this is to be sought in a consideration of the general physico-chemical characteristics of this class of substances. The colloids of protoplasm, by virtue of their slow diffusibility, inability to penetrate one another, slow penetrability or virtual impenetrability to many crystalloids,[1] and relative unliability to chemical change, form throughout the cell a substratum which admits of the persistence within the cell-limits of a high degree of non-homogeneity. This condition is evidently indispensable

[1] Seen in the non-permeability of colloidal membranes to many dissolved substances.

for the existence of the localized differentiations that constitute organized structure. Its physiological counterpart is seen in the fact that there are constantly taking place within the single cell a multiplicity of diverse and often contrary, yet simultaneous processes; these normally are so coördinated that the entire system is enabled to maintain itself in equilibrium and to carry on its vital functions within a certain more or less limited range of conditions. Thus the possibility of this coördinated differentiation of structure and function, which is perhaps the most distinctive peculiarity of living matter, appears to depend on the above properties of the colloids and on the special manner in which these substances are disposed within the cell.[1]

It is, therefore, to the conditions determining the disposition and state of aggregation of the colloids within the cell that we must look, when we attempt to explain the mode of origin of any given one of its structural features. Perhaps the most constant and distinctive of these features is the division into nucleus and cytoplasm. The proteids, which are the chief colloids of these two regions of the cell, are widely different in chemical nature; this implies a difference in their electrical properties, and with this is probably correlated the very typical segregation which they exhibit — the nuclear proteids forming a central aggregate which is almost always separated from the cytoplasmic proteids by a distinct membrane apparently impermeable to both. This seems the essential characteristic of the nucleated cell. A further peculiarity of the nuclear colloids is the remarkably definite disposition which they show at times; this is especially marked at the period of cell-division, when they form arrangements and go through movements of so characteristic a kind that the name *karyokinesis* has been given to the entire process. It is this peculiar mode of distribution of the nuclear colloids that I propose briefly to consider in the present paper: the movements and grouping of the nuclear colloidal aggregates, chromatic filaments and chromosomes, though apparently complex, are,

[1] For two important recent discussions of the part which colloids play in protoplasm, cf.: Hofmeister, "Die chemische Organisation der Zelle," Braunschweig, Vieweg, 1901; and Pauli, "Der kolloidale Zustand und die Vorgänge in der lebendigen Substanz," Braunschweig, Vieweg, 1902.

I believe, due essentially to a series of relatively simple conditions the nature of which I shall attempt to analyze below.

What then are the conditions that determine the disposition adopted by the chromosomes and the chromatic filament in the dividing cell? In the prophase of mitosis the chromatin[1] becomes disposed in so many cells in the form of one or more elongated deeply staining[2] filaments coiled within the nuclear membrane into a roughly spiral form, that the arrangement is regarded as typical. This is the well-known "spireme" stage. The spireme then segments into the definite number of chromosomes characteristic of the cell; the nuclear membrane disappears and the chromosomes are drawn into the equatorial plane of the cell; here they assume the arrangement known as the "equatorial plate" in which, it is to be noted, a distinct interval separates each chromosome from its neighbors. Each then divides, typically by a process of longitudinal splitting, into the two daughter-chromosomes which recede toward opposite poles of the cell; here later the daughter-nuclei are reconstituted from the two groups of daughter-chromosomes thus formed.

Both the adoption of this remarkable spiral arrangement and the manner in which the chromosomes become disposed in the equatorial plate are, I shall attempt to show, dependent mainly upon one simple physical property which the particles of chromatin possess in common with other similarly charged colloidal particles — namely, the property of mutual repulsion. It may now be considered as finally established that a colloidal substance in solution is in the condition of a more or less finely divided suspension, each particle of which is at a different electrical potential from the adjoining layer of liquid, *i. e.*, carries an electri-

[1] This is the cytological term; chemically this substance is undoubtedly nucleoproteid in nature; the chromosomes are probably similar to sperm-nuclei in their composition, as we may infer from the similarity between the micro-chemical reactions of the two. The nuclein of sperm-heads is relatively simple in composition and contains a large proportion of nucleic acid. For a general account see R. Burian in Asher u. Spiro's Ergebnisse der Physiologie, 3, 1894, I. Abtheilung, *Biochemie*, pp. 48–106.

[2] The increased depth of stain is an indication of increased acidity, *i. e.*, increased liberation of hydrogen ions; this involves an increase in the electrical negativity of the colloidal substance and consequently in the force with which adjacent portions of the filament repel one another.

cal charge. The sign of the charge is positive in the case of basic, negative in the case of acid bodies, since the former liberate negative (OH^-) ions, the latter positive (H^+) ions; the colloid particles themselves are thus left with free positive or negative charges respectively. All particles in a given colloidal solution, having like charges, must for this reason repel one another. Apparently the stability of a colloidal system depends upon this mutual repulsion of the particles of solute, since neutralization of the charge, as by the action of ions of opposite sign, results in an aggregation of the particles and the removal of the colloid from solution; to this action is to be ascribed the precipitating effect of ions.

If the particles composing the chromatic filaments and the chromosomes similarly repel one another — as in accordance with their colloid nature they must do — mutual repulsion will play an important part in determining the disposition which these structures adopt within the cell. The chromatin being an acid body must liberate hydrogen ions and become negatively charged, and this to a greater degree the higher its proportion of nucleic acid. I have elsewhere pointed out that sperm-nuclei, which are rich in nucleic acid, show a particularly strong tendency to travel with the negative stream when an electric current is passed through a solution containing them; this fact may be regarded as confirmatory of the view that the chromosomes, which in composition are probably very similar to sperm-nuclei, are likewise negatively charged bodies.[1]

Again the prevailingly central position of the nucleus within the cell — especially the cell about to undergo mitosis — indicates that there exists within the cell an influence tending to draw the nuclear colloids toward a central position. There are reasons for regarding this influence also as electrical in its nature; it seems highly probable that in the dividing cell the intra-nuclear colloids and the cytoplasmic colloids are opposite in their electrical properties[2]; they are certainly contrasted in their general chemical

[1] R. S. Lillie, *American Journal of Physiology*, 8, 1903, p. 273. Also BIOL. BULL., 4, 1903, p. 175.

[2] Proteids are amphoteric bodies, *i. e.*, may liberate a preponderance either of hydrogen or of hydroxyl ions according to conditions.

behavior; at all events they react toward acid and basic staining reagents in precisely opposite manners. This would indicate that the prevalent cytoplasmic colloids are preponderatingly[1] basic in character, *i. e.*, that their particles liberate hydroxyl ions chiefly, and are accordingly for the most part positively charged. It is conceivable that the hydrogen ions from the nuclear proteids and the hydroxyl ions from the cytoplasmic proteids, both of which ions have high migration velocities, find their way throughout the entire cell and unite with each other to form water. The colloids themselves, on the other hand, though of opposite sign, are unable so to unite, or can unite only partially, on account of the impossibility of their diffusing through one another; they are therefore left in the cell with free and opposite charges. Possibly a partial union of the nuclear and the cytoplasmic colloids may take place at the boundary between the two aggregates; it is known that colloids of opposite electrical sign will precipitate each other when their solutions are mixed, presumably by each effecting a neutralization of the other's charge.[2] Now it is a very remarkable fact that the nucleo-proteids of the cell are almost invariably separated from the cytoplasmic proteids by a well defined membrane, the "nuclear membrane," which is very possibly a precipitation-membrane formed at the surface of contact between the two oppositely charged colloidal aggregates. If this is so, it becomes intelligible why further neutralization of the charges is prevented and how each set of colloids is enabled to retain its charge in the presence of the other. The condition thus reached is that of a negatively charged aggregate, *viz:* the nucleus with its contained chromatin, in the midst of a positively charged field consisting of the numerous positively charged colloidal particles of the cytoplasm.[3]

The central position of the chromatin is now easily explained as due to the attraction which the aggregate of oppositely charged cytoplasmic colloids exercise upon it, the resultant effect of all

[1] Cf. R. S. Lillie, *loc. cit.*

[2] Biltz, *Berichte der deutschen chemischen Gesellschaft*, 37, 1904, p. 1095.

[3] This suggests J. J. Thomson's conception of the atom as a system of negative electrons moving in a field of uniform positive electrification. It would be strange if such a parallel should exist between the respective units of living and of lifeless matter.

these attractive forces being to draw the chromatin toward the center of the cell. On the other hand adjacent chromosomes or adjacent portions of the chromatic filament are by their own mutually repellent action exposed to an opposite influence which resists their approach toward one another. Hence the position of equilibrium, *i. e.*, the stationary position which they finally adopt, must be one where these two opposed sets of influences exactly balance each other.

The formation of the spireme and of the equatorial plate may then be ascribed to these two chief conditions, one the attractive influence that draws the chromatin toward a central position and tends to keep it there, the other the mutually repellent action of the chromatin particles themselves. The stationary position is one of equilibrium between these two opposed tendencies.

I should add here, in reference to the striking fact that the chromosomes at the metaphase occupy positions side by side *in a single plane* — that of the equator of the cell — that this remarkable peculiarity of disposition also remains to be accounted for. As yet, however, I know of no experimental facts bearing directly on this problem; and in the following explanation of the manner in which the equatorial plate is formed, I shall assume from the first that the chromosomes are free to move only in this single plane, without as yet attempting to explain why this is so.

Experimental.

If the conditions of spireme-formation and of equatorial plate-formation are similar to those suggested above, it should be possible to simulate these phenomena experimentally by making use of artificial filaments composed of mutually repellent units arranged in rows, and by subjecting these to the action of some centrally attracting force. The disposition and relative positions assumed by such filaments should, if the above hypothesis is correct, resemble those exhibited by the chromatic filament and chromosomes in the dividing cell.

In pursuance of this idea I have experimented with filaments consisting of rows of floating magnetized needles. The experiments of Alfred Mayer on floating magnets are well known to physicists; he studied many years ago the behavior of groups

of small magnetized needles which were floated by being passed through small discs of cork and placed on the surface of water in such a manner that all the magnets were vertical in position and similarly oriented, *i. e.*, with all the north (or south) poles uppermost. Such floating needles repel one another with a force inversely proportional to the square of their distance apart. If then over such a group floating with (for example) north poles uppermost a large bar-magnet is suspended in a vertical position with its south pole downward, all the needles are, by the horizontal resultant of the attractive force of the large magnet, drawn in toward a central position immediately below the latter; here they adopt arrangements of perfectly definite and regular configuration, whose exact form varies with the number of needles but shows great constancy for any given number.[1] The condition of stability of such systems is that the mutual repulsion which the small magnets exercise upon one another shall be exactly balanced by the centripetal attractive force due to the large magnet.

In their manner of grouping such floating magnets exhibit a close resemblance to the chromosomes of many equatorial plates, for example, those of the sea-urchin egg (seen in face). So far as I am aware, however, no attempt has hitherto been made to explain the grouping of chromosomes as due to conditions of the above kind. The following experiments have been designed with a view to testing this hypothesis.

To simulate the nuclear chromatic filament, the following procedure is employed: Small, similarly oriented, magnetized needles are strung at short and regular intervals (say 6 millimetres apart) along a delicate silk thread, preferably a single silk filament; each needle is passed through a small cubical piece of cork; the entire filament can then be floated on the surface of water with the needles vertical in position. When left undisturbed such a filament tends to be pulled out into a straight line by the mutual repulsion of its units; this form would be taken if the filament were ideally flexible and the supporting fluid devoid of viscosity, since then the average distance of the needles apart would be as

[1] Figures showing these arrangements will be found in any good text-book of physics, for example Ganot's.

great as possible under the conditions. Actually, however, the straight form is only approximated.

Such a filament may be caused to assume the spireme form as follows: A large bar magnet is suspended vertically over the filament with its north pole next the projecting south poles of the needles (or *vice versa*) and at a suitable distance from the latter. It is then found that the filament is drawn together by the attraction of the magnet into a more or less regular, close coil or spireme-like form, remarkably like that shown by the nuclear filament in the prophase of mitosis. Briefly, the explanation of this behavior is as follows: The filament is, by the attraction of the large magnet, confined within a limited space

FIG. 1. Artificial spireme from a sirgle filament; the small circles indicate the exact number and relative positions of the magnets.

just as the chromatic filament in the cell is confined by the nuclear membrane. Now, since mutual repulsion tends to prevent approximation of adjacent portions of the filament, the latter is forced so to dispose itself that, while occupying the greatest space possible under the conditions, the average distance between its adjacent portions is also as great as possible. This however leads inevitably to the production of the characteristic coiled or roughly spiral form which is the only one that satisfies these conditions, that is, in which the entire system is in equilibrium.

Figs. 1 and 2 are reproductions of exact drawings showing the form of the artificial spireme in each of two experiments, one with a single filament, the other with six separate filaments.

Their resemblance to the nuclear spiremes is evident. Such spireme figures may exhibit a great variety of forms; at present, however, I shall not attempt to enumerate these in detail ; a later paper will contain drawings showing the exact form assumed by

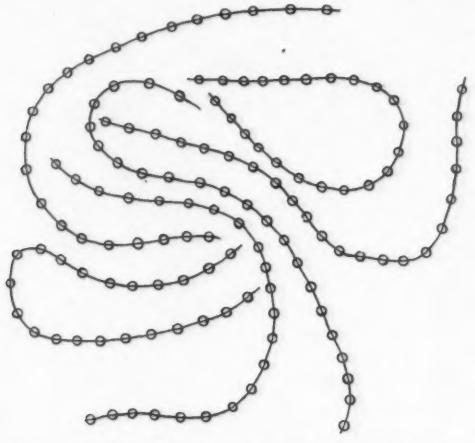

FIG. 2. Spireme from six separate filaments and disposition of individual magnets shown by the small circles.

the filaments under a variety of conditions: varying their number, size and structure, as well as the intensity of the central attractive force.

The formation of the equatorial plate may be simulated by the use of similar floating aggregates of needles, which in this case

FIG. 3. Arrangement shown by group of four chromosome models each with 9 magnets.

are strung along flexible wires capable of being bent into any desired shape. In this way the aggregate may be given the

form of any one of the various types of chromosome, and it thus becomes possible to study the effect which changing the form of the chromosomes has upon the configuration of the equatorial

FIG. 4. Arrangement shown by eight loop-shaped chromosome models.

plate. These chromosome models repel one another, and, like the isolated needles, they are found to exhibit very constant and regular configurations when subjected, as above described, to the attractive influence of the large magnet. The resemblance to

FIG. 5. Group of twelve loop-shaped chromosome models.

the configurations observed in the equatorial plates of actual cells is often very striking. I have made experiments with varying numbers of such chromosome-like aggregates and have

attempted to determine what the possible and stable configurations — that is, the conditions of equilibrium — are for any given number of chromosomes up to twelve.

Figures 3, 4 and 5 give exact representations of configurations shown respectively by groups of four, eight and twelve simple loop-shaped chromosome models. It should be added that the number of possible configurations becomes very great when the number of chromosomes is large. In the full paper drawings of a number of these will be given. I shall then consider the condition of their formation in greater detail.

It is my intention to investigate further the possibilities of this type of magnetic model. It is possible that such phenomena as the aggregation of the chromosomes in a single plane; their longitudinal splitting, and the separation of the daughter-chromosomes, may be simulated by means of such models. Much light may thus be thrown upon the mechanics of these characteristic phenomena.

It is to be noted that in experiments of the above type the filaments and chromosomes are arbitrarily confined in their movements to a single plane, that of the surface of the water. In this respect such models are incomplete, since, in the cell, movement is possible in all three dimensions. It seems clear, however, that, setting aside this partial and incidental limitation, these imitations of cell-phenomena are often surprisingly true to nature. *In so far as regards the mode of disposition* of the chromatic filaments and of the rows of magnets, the observed resemblance may be held to imply an identity in the essential determining conditions. These conditions are those of mutual attractions and repulsions, which are common to both systems. In other respects, it need scarcely be said, the two systems are widely different. Still, so long as science proceeds, as it has always done, by the progressive identification of apparently widely different things it will be necessary to isolate by analysis the features in which otherwise diverse phenomena agree, and to disregard, for the time being, their differences. We may with justification infer from the above that attractions and repulsions — here undoubtedly electrostatic in nature, since colloid bodies are concerned — play a most important part in determining the disposi-

tion of certain of the structural elements of the cell. How far such conditions have to do with form-determination in general is a further question, and one that stands in urgent need of thorough-going investigation. It may prove necessary to add electrostatic attractions and repulsions to the list of the conditions determinative of organic form and structure.

NAPLES, December 12, 1904.